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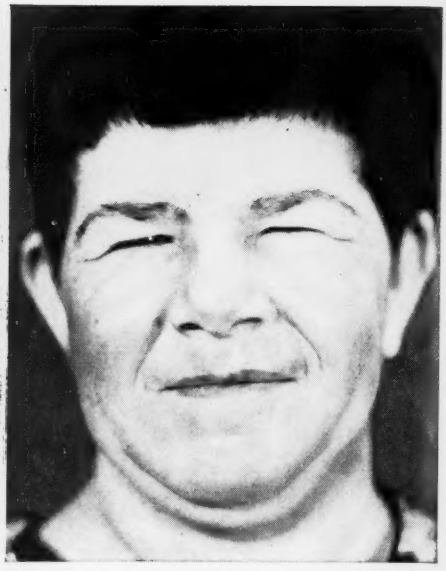

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CHROMOPHOBE PITUITARY TUMORS. I. DIAGNOSIS

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From the Lahey Clinic, Boston, Massachusetts

THIS review of our series of patients having chromophobe adenomas of the pituitary gland was undertaken to determine the relative frequency and nature of associated endocrine disorders and to evaluate the effects of medical, roentgen-ray and surgical treatment on these disorders as well as on the visual apparatus.

From the medical point of view, the problem of evaluating the presence or extent of endocrine deficiency presents itself in any patient whenever an enlarged sella is found roentgenographically. Roentgenograms of the skull are taken for various reasons in clinical practice and an enlarged sella is occasionally, and often unexpectedly, encountered. If well marked clinical signs of hypopituitarism are present, a presumptive diagnosis of chromophobe tumor is usually justified. When primary optic atrophy occurs, together with bitemporal visual field defects, the diagnosis is almost certain. Not all enlargements of the sella turcica, however, are due to chromophobe, mixed or eosinophil tumors, so that other causes of expansion of the sella must be considered. Chief among these are aneurysm of the internal carotid artery, suprasellar tumor, craniopharyngioma, cretinism, increased

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intracranial pressure from a variety of causes such as hydrocephalus, or intracranial tumors distant from the sella. In several cases we have noted an enlarged sella in females who were castrated before the age of 25. When it is realized that even marked visual field defects may result from verified chromophobe pituitary adenomas without gross evidence of endocrine deficiency, it is obvious that a careful survey of the endocrine status by special studies may be of value in any patients having enlargement of the sella turcica. Periodic observations before evidence of hormonal deficiency becomes clinically apparent should prove of interest.

Chromophobe tumors are often first suspected because of manifestations which are predominantly those of endocrine deficiency, but the majority are brought to light because of visual changes. If more patients with endocrine disorders were suspected of having a chromophobe tumor they would, in all probability, receive treatment earlier in the course of the disease and thus the large percentage of visual defects would, perhaps, be prevented. As just pointed out, however, serious visual loss may occur rapidly without any demonstrable endocrinologic signs or symptoms, so that even expertness in early recognition of the latter would fail to stave off serious visual change in an appreciable number.

In the present series of 164 presumed cases of chromophobe pituitary adenoma, the diagnosis was verified histologically in 105 cases, either at operation or at autopsy. The remaining 59 were "verified" by the fact that in each instance the patient had a typically enlarged, "ballooned" sella in association either with primary optic atrophy and bitemporal visual field defects or with unmistakable evidences of pituitary dysfunction, such as amenorrhea, changes in the skin and hair, cachexia, and so forth.

In studying the visual, neurologic and endocrinologic disorders recorded we have utilized the data obtained from the entire group of 164 patients. On the other hand, in the evaluation of the treatment of those who were subjected either to surgery alone or to roentgen therapy combined with surgery, obviously only the group of 105 cases of histologically verified chromophobe adenomas could be used. Of the remaining 59 patients there were 50 who had roentgen therapy without surgery and the results of this therapy have been recorded on the assumption that in these patients the diagnosis of chromophobe tumor was made because they had large sella turcicas as well as visual and other pituitary disturbances. The other 9 patients had large sella turcicas and pituitary dysfunction without visual manifestations and received only hormonal therapy.

INCIDENCE

The incidence of pituitary adenoma of all types in Cushing's series of 2,032 histologically verified brain tumors was 17.8 per cent (1). This is a

higher incidence than that noted at other centers, because of Cushing's interest in and manifold investigations of pituitary disorders. Grant (2), in analyzing 1,001 verified intracranial tumors over a ten-year period, found 71 or 7 per cent to be pituitary adenomas. In our series at the Lahey Clinic, there have been 1,467 verified brain tumors from 1933 through 1949. Thus, the 105 verified chromophobe pituitary adenomas represent 7.1 per cent of all our brain tumors. If one were to add the 16 chromophil tumors which have been verified by operation, the total verified adenomas would be 8.2 per cent. Henderson (3) reviewed Cushing's results in 338 verified pituitary adenomas, of which 260 (76.9 per cent) were chromophobe adenomas. Of our total of 121 verified pituitary adenomas, the 105 of the chromophobe variety represent 86.8 per cent.

NATURE AND SPREAD OF PITUITARY ADENOMA

Adenomas of the pituitary are of four types—chromophobe, acidophil, mixed type and basophil. Although a few cases of mixed tumor have been included, this paper deals chiefly with the first type, the chromophobe adenoma, composed of nongranular cells which are nonsecretory.

Although adenomas occasionally are believed to begin with a diffuse hyperplasia of chromophobe cells scattered throughout the anterior lobe, the majority usually begin as small discrete adenomas. Costello (4) has found small encapsulated collections of cells, "subclinical" adenomas, more commonly than generally would be expected. He reported that 25 per cent of 1,000 cases coming to autopsy had adenomas that ranged from microscopic size up to 0.5 cm. in diameter, but produced no symptoms. As the normal pituitary gland occupies about 50 per cent of the volume of the sella turcica there exists at first adequate room for asymptomatic expansion. As the adenoma enlarges, the normal pituitary tissue is compressed; and when it is partially or wholly destroyed, varying degrees of hypopituitarism result. The adenoma may, by further expansion, stretch the thin diaphragma sellae upward, with resulting pressure on the optic nerve and visual defects. On the other hand, it may extend through its anatomic barriers in one or more directions. It may grow downward through the floor of the sella, filling the sphenoidal sinus and occasionally presenting itself as a large mass in the nasopharynx. By rupture through the diaphragma sellae, it may bulge into the postchiasmal space against the optic nerves and, as it becomes larger, expand into the floor of the third ventricle, eventually resulting in internal hydrocephalus. Small protrusions of the tumor may occur through openings in the diaphragma sellae and impinge discretely on one or the other optic nerve. The adenoma may spread out laterally, expanding under or into one or the other temporal lobe, where it may be silent until it reaches a large size. Then it may produce

bizarre neurologic symptoms not commonly seen with pituitary tumors.

When the adenoma has spread beyond the sella into the cranium, it is clearly outlined by a smooth, soft capsular condensation of its own substance (3). This prevents adherence and infiltration into surrounding neural structures and hence complete surgical removal of this extrasellar portion may at times be possible. Some intracranial extensions, however, are extremely large, or they may surround fixed structures in the vicinity of the sella, such as the internal carotid artery or oculomotor nerves, without affecting their function. In such instances complete removal of the tumor is impossible.

Just how an adenoma which has enlarged to a sufficient size breaks out of the sella turcica and spreads intracranially or elsewhere is not entirely understood, but this process is thought to be dependent upon the relative strengths of the diaphragma above and of the roof of the sphenoidal sinus below. In a few instances the tumor grows only upward through the diaphragm and a large suprasellar tumor is formed without radiologic evidence of destruction of the sella.

In most cases the classic visual defect, bitemporal hemianopsia, occurs with chromophobe adenoma as the tumor impinges on the optic chiasm. The visual field defects usually begin as scotomas or cutting-in of the superotemporal quadrants; however, other defects which are detectable on visual field examination may be present. Homonymous hemianopsia may occur if the tumor expands laterally out of the sella, compressing the optic tract behind the chiasma; or if it grows into the temporal lobe, affecting the optic radiation. In a few cases, one eye may be blind, without the opposite eye being affected. This usually indicates a large tumor that has expanded anteriorly and is impinging on the optic nerve in front of the chiasma. A few instances of bilateral blindness have been observed. These usually are indicative of large adenomas of long standing. We have seen several cases in which, despite an unusually large sella as shown by roentgenogram, no visual defect could be demonstrated. It is also possible for an intrasellar tumor to eliminate pituitary function completely, without gross enlargement of the sella.

An important criterion in the diagnosis of chromophobe adenoma, whether or not an expanded sella is present, is a progressive defect in the visual fields. If this can be determined by the history, so much the better; but when such defects are found objectively, roentgen ray or operative therapy is indicated, according to the extent of visual loss.

Relief of pressure on the optic nerves usually results in visual improvement but vision may be permanently damaged if the adenoma compresses the nerves against the laterally placed carotid artery. By this means, one

or both optic nerves may suffer irreparable damage. Pressure against the artery accounts for the lack of improvement after operation in some cases in which the adenoma was only of moderate size.

Chromophobe tumors are usually benign but a certain number of them recur after surgical removal. There appears to be a wide difference in the rate of growth and the ability to form again after surgery. A few cases of malignant adenoma have been reported by Bailey and Cutler (5).

Many of the adenomas in this series were cystic (Cushing's series, 17 per cent). The cysts are of two types: 1) the more common hemorrhagic type, secondary to hemorrhage into degenerated areas of the adenoma, and 2) those cysts which contain xanthochromic fluid formed by the chromophobe cells. The cysts are generally unilocular and walled by a layer of tumor of varying thickness. Although it is impossible to distinguish the cystic type before operation, suggestive evidence is provided by acute visual symptoms due to a hemorrhage causing sudden tension on the already slowly stretched optic nerves. Although cystic tumors might be thought to have a favorable surgical prognosis because of the ease of aspiration, and the complete decompression of the optic nerves, they (especially xanthochromic cysts) tend to recur quickly and fill with fluid after operation.

There is a type of adenoma described by Bailey and Cushing (6) which contains a few acidophilic granules around the periphery of the cells. Clinically, Bailey and Cushing termed these cases "fugitive acromegaly" because there was evidence of hypopituitarism superimposed on previous but usually slight acromegalic skeletal changes. These tumors are important because they frequently grow to a large size. A few cases of this type are included in this series.

AGE AT ONSET

In 16 cases the adenomas began before the patients were 20 years of age, the youngest being 13. In 87 (61 per cent), the patients were between 30 and 60 years of age; and in 12 cases, past 60 years of age.

In many instances, a long period of time elapsed after the onset until the patient sought medical advice. This is borne out by the fact that 45 patients had symptoms for at least five years and 19 had symptoms for more than ten years before admission to the clinic or hospital. As will be demonstrated later, minor symptoms occur earlier than major symptoms and are usually overlooked.

SEX

In our series there were 81 women and 83 men. Approximately 70 per cent of each group were married.

SYMPTOMATOLOGY

The clinical manifestations of pituitary chromophobe tumor may arise in two ways. In the first, the symptoms and signs result from pressure, extension, or both, of the tumor into adjoining neural structures, especially

TABLE 1. SYMPTOMS COMPLAINED OF IN ADDITION TO HEADACHE AND VISUAL DISTURBANCES

Central Nervous System		*General*	
Drowsiness	20	Indigestion	2
Insomnia	6	Anorexia	4
Mental slowness	3	Nausea	4
Impaired memory	5	Abdominal distention	1
Disorientation	3	Vomiting	1
Lack of insight	2	Gain in weight	3
Inability to concentrate	1	Anemia or pallor	11
Mental depression	1	Cold sensitivity	4
Inversion of sleep rhythm	2	Swelling of forehead	1
Personality deterioration	3	Puffy eyes	2
Visual hallucinations	2	Loss of ambition	1
Tremor and weakness	3	Weakness	11
Unilateral	2	Fatigability	24
Bilateral	1	Apathy	4
Hemiplegia	1	Pressure and pain back of eyes, nose and between ears	1
Maniacal spells	1	Fall in blood pressure	1
Convulsions	3	Polydipsia and polyuria	1
Unconscious spells	2	Pain	5
Syncope	2	Generalized	1
Uncinate attacks	2	Back	2
Deafness	4	Arm	1
Tinnitus	3	Shoulder	1
Vertigo	3	Pain around eye	8
Somnambulism	1		
Lisping speech	1		
Anosmia	1		
Paresthesias	2		
Sexual			
Failure to ejaculate	2		
Impotence	1		
Amenorrhea	20		
Primary	3		
Secondary	17		
Loss of sexual hair	21		
Loss of libido	1		
Sterility	1		
Hot flashes	3		

the optic chiasm. In the second, the clinical picture arises from the hormonal insufficiencies caused by the tumor pressing on or destroying the remaining functioning pituitary tissue, or the hypothalamus, or both. Table 1 lists, in order of frequency, the more important complaints as noted in this series.

Visual disturbances

Visual disturbance was the presenting complaint in 95 cases (about 58 per cent); but on examination visual disorders were found in 132 patients or 80 per cent of the total, varying from a single scotoma to complete blindness. Blurred vision and loss of temporal vision were the most frequent visual complaints (Table 2).

TABLE 2. VISUAL DISTURBANCES (SUBJECTIVE)

Blurring		74
Half vision (blinders)		29
Sight in one eye		12
Blind		2
Diplopia		15
Ptosis (unilateral)		3
Strabismus (recent)		1
Scotoma		1
Scintillating		1
		138
No eye symptoms	18	

The majority of patients with symptomatic chromophobe adenomas first seek advice because of visual symptoms which usually indicate that the tumor has broken out of the sella. A careful history may often reveal that other minor symptoms have preceded the visual changes which, had they been heeded, might have initiated earlier treatment. Thus, in some instances at least, irreparable visual damage would have been prevented.

Headaches and neurologic symptoms

In addition to visual disturbances, other symptoms from local pressure occur. Headaches, the second most frequent symptom, occurred in 66.8 per cent. These were nondescript for the most part, varying in location, frequency and severity. In 9.1 per cent of cases the headaches simulated migraine and may well have been migraine, with no relation to the tumor.

Neurologic manifestations occur occasionally and are indicative of a large adenoma that has expanded into the base of the brain. The symptoms of drowsiness, stupor or coma, insomnia, convulsions, personality changes,

impaired memory, visual hallucinations and varied paresthesias are similar to those produced by cerebral pressure from any cause.

Pressure on the hypothalamus may result in amenorrhea; it has been observed in several patients in whom urinary hormone assays revealed normal quantities of FSH and 17-ketosteroids. The presumption is that LH is not released, but supportive data are lacking because satisfactory tests for luteinizing hormone are not available.

Hormonal insufficiency

Clinically, endocrine deficiency occurs following pressure upon, or destruction of functioning pituitary tissue; thus, pituitary function may be normal, deficient or completely absent, depending upon the extent of the process. Underfunction is manifested by secondary depression of the function of the peripheral endocrine glands especially the thyroid, adrenals and gonads. Occasionally, there is a clinical pattern that appears to be mainly of a uniglandular type but closer examination usually reveals concomitant evidence of depression of other glands to a lesser degree. This may be due to a predominant deficiency of one of the pituitary hormones or to the inability of an end organ or gland to respond to reduced pituitary stimulation (Cases 1 and 2).

Chromophobe tumor mimicking Addison's disease

Case 1. The patient, a woman aged 70, was first seen in August 1933, complaining of vomiting, weakness and faintness of one year's duration. Other complaints included stupor, disorientation, headaches and dry skin. On examination, there was no pigmentation. Systolic blood pressure was recorded as 120 mm. of Hg. She was treated successfully with Eschatin and sodium chloride.

The patient's course was followed until 1937, during which interval there was good response to treatment. At that time, she was admitted to the hospital because of weakness and lethargy. A very large sella was found by roentgenologic examination. The basal metabolic rate was −17 per cent; fasting blood sugar level, 91 mg.; plasma cholesterol, 260 mg. per 100 cc.; and blood pressure, 116 mm. systolic and 90 mm. diastolic. Visual field defects were found on perimetry. The diagnosis of pituitary adenoma with cachexia and secondary adrenal insufficiency was then obvious. She responded to treatment but was readmitted to the hospital October 22, 1938, and died in what appeared to be an addisonian crisis. Postmortem examination showed pituitary adenoma and adrenal atrophy.

Clinical myxedema with enlarged sella; question of chromophobe tumor

Case 2. A 41-year-old married woman with children complained of weakness and fatigue of two years' duration. She had passed through the menopause seven years previously, but had had hot flashes for only a few months; libido was lost. She was sensitive to cold.

The skin and hair were dry. Speech and movements were slowed and the patient's voice was thick. Her appetite was poor. Pubic hair was practically absent and body and axillary hair completely absent. The blood pressure was 96 mm. systolic and 80 mm.

diastolic, plasma cholesterol level 309 mg. per 100 cc., and basal metabolic rate −30 per cent. The clinical impression was myxedema. A roentgenogram of the skull revealed an enlarged sella turcica. The results of the water test were positive. There were no visual field or acuity changes. She was treated with thyroid and salt and then pellets of desoxycorticosterone and testosterone were implanted. Several years later the salt and pellets were discontinued and thyroid was continued, without return of signs or symptoms. Recently thyroid was discontinued, resulting in clinical myxedema, which disappeared on resuming thyroid. This case could represent primary myxedema with secondary changes in the pituitary.

Amenorrhea

One of the most frequent symptoms of chromophobe adenoma in females under the age of 42 is amenorrhea.

Chromophobe tumor with amenorrhea as a presenting symptom

Case 3. A married woman, aged 30, had had amenorrhea for ten years and headaches for five years. The headaches were usually mild but occasionally severe and throbbing in the occipital region. When she was 23 years of age thyroid deficiency was suspected because of a low basal metabolic rate (−24 per cent) and she was given thyroid without improvement. She claimed that her sex life was normal.

The patient's appearance was normal; there was no anemia. Pubic and axillary hair were normal; she shaved the axillary hair every two months. The basal metabolic rate was −13 per cent and blood pressure 110/70. The plasma cholesterol level was 149 mg. per 100 cc. The sugar tolerance curve was normal. No FSH was found in the urine (unconcentrated); 17-ketosteroids were 14.9 mg. per twenty-four hours. The adrenal water test gave negative results. The sella was enlarged (20 by 15 mm.) with depression of the floor into the sphenoidal sinus. Visual fields were normal. Two series of roentgen-ray treatments were followed by relief of headache. Amenorrhea persisted unless she was given periodic courses of estrogen orally followed by anhydrohydroxyprogesterone bucally.

Amenorrhea occurred in 40 cases before the age of 42 (51 per cent) and was the presenting complaint in 16 cases. It has been generally considered that without the presence of amenorrhea, the existence of a symptomatic chromophobe tumor is unlikely. We have observed 3 cases, proved histologically, in which visual field defects occurred in the presence of normal menstruation. On the other hand, amenorrhea may occur without detectable evidence of adrenal cortical or thyroid deficiency. Urinary FSH may even be present in normal or excess quantities. This phenomenon is often considered to result from pressure upon the hypothalamus, which prevents release of pituitary gonadotropins; however, the presence of urinary follicle-stimulating hormone in some cases is contrary to this hypothesis.

Amenorrhea, especially before the age of 42, without hot flashes, should arouse suspicion of a chromophobe adenoma, and such was the case in 25 of our series. On the other hand, 12 patients had amenorrhea with hot flashes, the latter being the chief complaint in 3 patients. In 6 of the 12 cases with amenorrhea and hot flashes, the diagnosis of chromophobe

tumor was confirmed histologically. In 2 patients the flashes persisted despite irradiation and operation. A menopausal urine (excess FSH) was demonstrated in a few of these cases but more frequently a low or absent titer of FSH and 17-ketosteroids was found in the urine. The occurrence of hot flashes under these circumstances leads one to speculate as to their cause. The following course of events appears plausible: As the adenoma enlarges within the sella, pituitary gonadotropins are gradually decreased. Amenorrhea then ensues and persists, the ovaries becoming atrophic and eventually incapable of stimulation. When the adenoma breaks through the capsule, pressure within the sella is released allowing, in some cases at least, a resumption of gonadotropin secretion, since the ovaries are now atrophic and excess gonadotropins are released as in a natural menopause. Other explanations, of course, are possible.

Libido sexualis

The loss of libido and potentia, especially in men, is an important symptom. As in the case of amenorrhea in the female, investigation should include roentgenograms of the skull. It is noteworthy that only 1 male patient complained of loss of libido and another of inability to ejaculate; nevertheless, on questioning 36 patients, it was found that 72 per cent had noted a decrease in this function (half were under 40 years of age). This lack of concern about loss of libido is in contrast to that found in other forms of impotence in which hormonal deficiencies are usually not to blame. Loss of aggressiveness and other personality changes which occur with loss of interstitial cell function are not infrequent.

Sterility and fertility

Several women whose chief complaint was sterility, and in whom amenorrhea existed, were shown by roentgenogram to have enlarged sellae. They almost certainly had chromophobe adenomas, which were the cause of their reduced ovarian function. Fertility may persist in the male or female with a large chromophobe tumor (Case 4). Fifty-seven of the 97 married patients had children before the onset of their complaints. Two men were each the father of 11 children.

Fertility in a male with chromophobe tumor

Case 4. A man, aged 30, who had been married five years, complained chiefly of headaches, and moderate fatigue and weakness of seven years' duration. He was the father of one child and his wife was pregnant at the time of his examination. His fundi were normal but there was a slight loss of the temporal field of the left eye. On roentgenologic examination, the sella was enlarged with intact clinoids, its area being 201 sq. mm. Roentgen-ray treatment afforded relief of his symptoms. He died at home one year later of "meningitis"; autopsy was not performed.

Normal menstruation and pregnancy with chromophobe tumor and optic atrophy

Case 5. This woman was first seen at the Lahey Clinic in 1944 when she was 28 years old. In 1936 she had been operated on elsewhere for removal of a cyst pressing on the optic nerves, with subsequent restoration of vision. She had had two children by means of cesarean section. The second delivery in 1943 was followed shortly by diminished vision, until she was blind in the left eye. She was given 10 roentgen-ray treatments. There was no change in the amount and rhythm of her menstrual periods, except when she was pregnant.

On roentgenologic examination, the anterior clinoid was almost completely absent, with depression of the floor of the sella. A ventriculogram showed a porencephalic cyst in the left frontal area. Operation was performed on May 19, 1944, at which time as much tissue as possible was removed, although the tumor extended back under the hypothalamus. The pathologic report disclosed a degenerated pituitary adenoma. She received irradiation after operation. Useful vision was regained in the right eye and there was some return of vision in the upper nasal quadrant of the left eye. In July 1945, pregnancy ensued and was interrupted; she was again pregnant in February 1946; abdominal hysterectomy and sterilization were performed. At that time, extensive atrophy of the left disk was present with no vision; and on the right, vision was 5/200 with only a small nasal hemianoptic field remaining.

Anemia and fatigue

There are several other symptoms which occur often enough to be significant. "Fatigue" was complained of in 24 cases. A striking pallor or anemia was present in 11 cases. Both of these symptoms were present in Case 6.

Anemia as the first sign of chromophobe tumor

Case 6. The patient, a man aged 23, came to the clinic in June 1940. The blood pressure was 98 mm. systolic and 58 mm. diastolic. His weight was 117½ pounds and his height was 67 inches. He had lost 10 pounds in one year and had anemia and fatigue since a nasal operation four years previously. He had been treated with iron without improvement.

The basal metabolic rate was normal. A roentgenogram showed a normal chest. The hemoglobin was 11.7 Gm. and erythrocytes numbered 3,970,000. He was given iron therapy for one month with no improvement. Five years later (June 1945) he was discharged from the Armed Services, where he had received roentgen and testosterone therapy because of a chromophobe adenoma which was first suspected because of the sudden onset of visual change. The basal metabolic rate was −30 per cent and a visual field defect was found. The hemoglobin was 13.2 Gm. and the plasma cholesterol level, 185 mg. per 100 cc.

The patient was given further roentgen-ray therapy. Testosterone pellets were implanted and he also received benzedrine. In March 1948 his general health was better. Later he took methyltestosterone, 10 mg. daily, with increased libido and erections every four or five days. There was less fatigue and drowsiness. He shaved every two days. Axillary hair was absent and pubic hair was scanty. The visual fields were normal, with 20/15 vision. An examination of the ejaculate showed no spermatazoa. The sella measured 260 sq. mm. The hemoglobin was 13.2 Gm., and erythrocytes numbered 4,870,000.

A testicular biopsy specimen showed marked hyaline changes in the basement membrane; Sertoli's cells were present and a few spermatogonia; no Leydig cells were seen.

The presence of anemia on first examination was considered a reasonable explanation of fatigability. Further questioning later on revealed sex function to be absent at the time of the first examination; as usual with these cases, this fact was not complained of, the patient attributing the loss of libido to anemia and fatigue.

Gastro-intestinal symptoms

No striking gastro-intestinal symptoms were observed. Nausea and vomiting, which are sometimes observed with brain tumors, are very rarely noted in pituitary adenoma. Anorexia is not infrequently present when there is marked hypopituitarism. Polyuria and polydipsia were noted in 5 cases but in only 1 case was it distressing enough to warrant therapy. These symptoms of disturbed posterior pituitary or hypothalamic function were seen more commonly after operation; they were usually transient, but persisted in 2 cases.

PHYSICAL FINDINGS

The main changes occur in the skin, sexual hair, genitalia and fundi. The skin was notably soft and smooth in 52 cases. A characteristic fine wrinkling of the forehead may often be seen. A striking pallor or sallow appearance, out of proportion to the degree of anemia, was recorded in 41 cases. No characteristic pigmentary changes occurred, but patients with weight loss had a brownish pigmentation from contraction of the skin. Black freckles and melanin deposits in the skin folds, over pressure points, and on mucous membranes were not found in patients in whom the adrenal insufficiency was secondary to the hypopituitarism, thus differing from the pigmentary changes of primary adrenal insufficiency (Addison's disease).

Weight and body contour

Changes in weight may or may not be marked. In 38 cases no change of weight was observed. In 27 cases there was a gain of weight, with the maximal gain of 87 pounds following the onset of symptoms; and in 23 cases a loss of weight occurred, with a maximum of 84 pounds. As will be pointed out later, adiposity when and if it occurs, is usually characterized by its female distribution.

Several male patients had feminine body contours, flabby subcutaneous and muscular tissues, and tapered fingers (which we believe are due to fat hands and are not in any way a pituitary sign). The distribution of fat is usually feminine, which in males we believe is due to a reduced secretion of male sex hormone from the testes rather than to a direct effect of the pituitary tumor on fat distribution. This distribution of fat resembles that

seen in male castrates, in whom body contour may be restored to normal with adequate male sex hormone therapy.

Hair

In hypopituitarism, the sexual hair is characteristically reduced or may be completely absent. This applies to the body, acral, axillary, pubic, facial and aural hair. The scalp hair in 31 cases was recorded as scant, fine and silky, and in some cases there was partial loss of eyebrows. In patients with marked secondary hypothyroidism the scalp hair is of such poor texture that in females it is "hard to manage" and will not take or hold a permanent wave. The beard in males is affected frequently, with a reduction in the amount and the rate of growth, requiring less frequent shaving. In some instances, when axillary hair is cut it fails to grow or may regrow very slowly and sparsely, if at all. In 28 cases, skin and hair were recorded as being normal.

A current concept is that the adrenal cortex maintains and controls the basic sexual hair growth in both sexes; but in the male, the beard and upper part of the pubic hair are primarily under testicular control. In hypopituitarism, the latter appears to be first to decrease.

In females we have observed that sexual hair may be present and, if cut, may regrow, in spite of a low 17-ketosteroid output and positive findings with the adrenal water test; we have not, however, measured such regrowth quantitatively. Furthermore, in true Addison's disease the loss of axillary and pubic hair is infrequent, although in rare instances it may almost entirely disappear. This is not in complete accord with current theories and leads one to speculate that sexual hair may be stimulated and maintained in part directly from the pituitary gland.

Genitalia

The genitalia often reveal changes. This is more evident in males, who may have soft, small testes. Testicular biopsy of these hypoplastic testes reveals few or absent Leydig cells and tubular hypoplasia, with little or no spermatogenesis. Hyaline change may appear along the basement membrane. Only 1 patient, aged 63, had symptoms referable to the prostate gland, which was palpably enlarged; transurethral prostatectomy had been performed three years before the onset of visual symptoms. On examination, 2 other patients had enlargement of the prostate gland, a condition which was considered to have been present before the onset of pituitary symptoms.

Arthritis

It is noteworthy that osteoarthritis, particularly Heberden's nodes, is rare in hypopituitarism of long duration, in contrast to acromegaly, in

which it is frequent. Rheumatoid arthritis, too, is unusual, although there is reason to suspect that collagen disease may be present oftener than hitherto believed.

Blood pressure

The systolic blood pressure levels varied widely. In 63 cases, the systolic pressure was below 120 mm. of mercury and in 13 cases below 100 mm. Considering hypopituitarism to be a disease of middle life, it is apparent that the blood pressure in this disorder tends to be low and comparable to that in Addison's disease. The observed level is probably dependent in some degree on the blood pressure before the onset of symptoms.

In 21 patients, nearly all over 55 years of age, the systolic blood pressure was over 140 mm. of mercury. In 11 of these cases the pressure was greater than 170 mm. and in 2 cases readings of 216 mm. and 230 mm. were recorded. In all but 2 instances the presenting complaints were visual disturbances or headaches or both. On examination only slight or no evidence of hypopituitarism was found, except in 1 case of a 73-year-old widow who exhibited a moderate degree of hypopituitarism with a blood pressure of 156 mm. of mercury (no previous readings available). Basal metabolic readings in these cases ranged from −2 to −37 per cent. Nine patients were operated on for removal of the tumor, and the remainder received roentgen irradiation. There was no record of the blood pressure rising following such treatment.

In some cases operation was followed by marked reduction in blood pressure. For example, in a 65-year-old woman with systolic blood pressure levels between 170 and 180 mm., known preoperatively for three years, the pressure following surgical removal of the tumor was 100 to 105 mm. of mercury. Marked, temporary hypopituitarism also developed.

Another interesting example of the effect of diminished pituitary function on hypertension is illustrated in Case 7.

Chromophobe tumor, hypertension, and presumably hemorrhage into the tumor, followed by bitemporal visual field defects, hypotension and hypopituitarism

Case 7. In a woman, aged 65, known to have hypertension (readings over 200 mm. systolic and 100 mm. diastolic) for five years, there suddenly developed, four years before admission, severe headache and bitemporal hemianopsia. This was followed later by weakness, fall in blood pressure to 110/80 and loss of axillary and some pubic hair. Partial visual field recovery took place spontaneously. A roentgenogram showed an enlarged sella; and an oxygen encephalogram revealed a tumor which bulged upward. The clinoids were intact. It was thought that aneurysm was not present. Roentgen-ray therapy and medical treatment with testosterone, thyroid, and implantation of desoxycorticosterone pellets improved her general condition. The patient voluntarily returned for a second implantation of pellets.

Visual apparatus

Ophthalmoscopic examination of the optic nerve head is, of course, important in all patients suspected of having a pituitary adenoma. In only 34 cases in this series were the fundi recorded as normal. The changes usually seen are those of primary atrophy of the optic nerve, namely, a clear sharp edge, pallor and deepening of the physiologic cup. Occasionally, slight edema of the disk or blurred edges may be seen, and in 1 case choking of the nerve head was observed. This was interpreted as evidence of increased intracranial pressure secondary to a large tumor, but in a recent case not included in this series no such pressure was demonstrated at operation.

Examination of the visual acuity and the visual fields with the perimeter should be carried out in all suspects. In 132 cases (80 per cent) the visual acuity was impaired in one or both eyes. The visual fields were regarded as normal in only 28 cases (17 per cent). Four cases showed a slight visual field defect in the upper temporal region that was found only on careful perimetry. The visual field defect is usually confined to loss of the temporal fields but in a small number of cases infringement of the nasal fields was observed.

Blood

The hemoglobin level and red cell count were usually reduced, as shown in Table 3.

TABLE 3. BLOOD FINDINGS

	Males	Females
Average hemoglobin*	84.9%	89.4%
Range of hemoglobin	65 to 105%	60 to 104%
Average red blood cell count (millions)	4.3	4.39
Range of red blood cell count (millions)	3.3 to 5.4	3.46 to 5.57

* Based on male standard, 15.6 Gm. per 100 cc.; female standard, 14.0 Gm. per 100 cc.

The exact cause of this normocytic anemia is not known. Undoubtedly it is the result of hypopituitarism and probably reflects the degree of hormonal deficiency, especially that of the secondary hypoadrenocorticism. The bone marrow has been reported as hypoplastic in hypophysectomized animals. When hemoglobin values of below 65 per cent are found, other coincidental causes of anemia should be suspected and sought.

THYROID FUNCTION

In the presence of hypopituitarism, the thyroid gland becomes hypoplastic, with reduced function. This should be manifest in a low basal

metabolic rate and high plasma cholesterol level, but unless there is a predominant thyroid deficiency producing in some cases clinical myxedema, the cholesterol values are not likely to be exceptionally high. The basal metabolic rates in our cases were low, averaging −20 per cent. In the 86 cases tested, only 27 patients had a basal metabolic rate reading above −10 per cent.

The plasma cholesterol level was determined in 76 cases and averaged 230 mg. per 100 cc. (slightly above the upper limits of normal), with a range from 144 to 394 mg. per 100 cc. There appeared to be no direct correlation between the cholesterol level and the basal metabolic rate determination. When the plasma cholesterol is high, as it usually is in primary thyroid myxedema, it may be because there is a preponderant deficiency of thyrotropic hormone, although the extreme variations in apparently normal individuals must not be lost sight of. The thyroid gland has a low level of function independent of the pituitary and it may be that this phenomenon prevents the appearance of classic primary thyroid myxedema in the majority of cases of panhypopituitarism associated with chromophobe tumor. To produce myxedema in otherwise normal persons, all functioning thyroid tissue must be removed. Experience has shown that small doses of thyroid substance (0.5 grain or less) will cause the main signs of myxedema (dry skin, edema and so forth) to fade, even though the optimal dose is two to three times as much.

As might be inferred from the foregoing, the patient with hypopituitarism, despite the low metabolic rates, does not usually appear myxedematous, having a soft, smooth skin and being mentally alert and active. There are instances, however, in which the clinical appearance of the patient and other signs and symptoms simulate the true myxedema of primary thyroid deficiency. Visual field impairment or decreased sexual hair should direct attention to the pituitary rather than to the thyroid gland. All cases of idiopathic or spontaneous myxedema should have roentgenograms of the skull in order to rule out a pituitary adenoma. It must be remembered that hypothyroidism causes a slight hypertrophy of the hypophysis, which is seen chiefly in young cretins. In our experience the adrenal water test has always given positive results in cases of myxedema secondary to panhypopituitarism but negative results in all cases of primary thyroid myxedema that were tested. It is distinctly possible that long-standing primary myxedema may produce pituitary deficiency, which, in turn, may manifest itself by a positive response to the water test and a negative eosinophil response to epinephrine (Case 2).

CHEMICAL DATA

The fasting blood sugar level was usually low but within normal range, averaging 84.9 mg. per 100 cc. in 49 cases (range, 73 mg. to 137 mg. per

100 cc.). In only 3 cases did the blood sugar curve rise temporarily above the arbitrary diabetic level of 170 mg. per 100 cc. during the glucose tolerance test, and in no instance was there glycosuria. In the few patients who had simultaneous inorganic blood phosphorus determinations during the glucose tolerance test, there appeared to be normal utilization of glucose in the body tissues as shown by a fall in phosphorus. In cases of secondary adrenal insufficiency, just as in Addison's disease, a low glucose curve was observed.

Severe hypoglycemic reactions may occur (although such has not been identified in any of the cases in this series) and insulin sensitivity is increased in cases of hypopituitarism. In none of the cases was a history obtained of diabetes which had been ameliorated by development of hypopituitarism, a state found in the Houssay animal.

The serum inorganic phosphorus level as an index of growth hormone activity should be of interest in cases of mixed tumor in which slight acromegalic changes are taking place. We have not had the opportunity to make these determinations, since the cases of fugitive acromegaly are recognized long after activity has ceased.[1] A fasting blood phosphorus level consistently below 4.0 mg. per 100 cc. appears to be an aid in ruling out active acromegaly. In the chromophobe cases in which determinations were made, the average was 3.8 mg. per 100 cc.

The adrenal water test is inexpensive, simple to perform and easy to interpret. We consider the response to be positive if none of the day specimens of urine exceed the overnight volume. In our series, the results of the water test (Kepler-Robinson) were positive in 10 cases and negative in 8 cases. In the 10 cases there were other evidences of hypopituitarism, whereas in the 8 cases there was scanty evidence, with the exception of amenorrhea. It is to be remembered that the response to the water test may be positive in other conditions and occasionally in apparently normal individuals.

Hormonal studies are interesting and may contribute to the establishment of the complete diagnosis, but are time-consuming and expensive. The 17-ketosteroid urinary excretion averaged 11.3 mg. per twenty-four hours, with a range from 1.0 to 31.5 mg. per twenty-four hours. A low 17-ketosteroid excretion is indicative of lowered adrenocortical function in the female and of both testicular and adrenocortical function in the male. Values usually run parallel to the degree of pituitary deficiency although in some cases the findings are surprising.[2] In well marked hypopituitarism,

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[1] A recent case with minimal acromegalic changes but otherwise clinically a typical instance of chromophobe adenoma, showed several fasting inorganic phosphorus values between 5 and 6 mg. per 100 cc., which might be interpreted as indicating a mixed tumor.

[2] A young man with classic signs of hypopituitarism and bilateral hemianopsia was repeatedly found to have a urinary 17-ketosteroid excretion above 30 mg. per twenty-

values of from 1 to 3 mg. per twenty-four hours are not uncommon. Unfortunately, values of from 3 to 4 mg. are also found in primary myxedema, so that the determination is of limited value in differentiating between the two conditions except when values are around 1 mg. On the other hand, the water test provides a fairly reliable guide in this connection. Urinary follicle-stimulating hormone[3] (Prolan) of the anterior pituitary, as determined in 14 cases, was reduced and was present qualitatively in only 1 case. Estrin determinations in amenorrheic females under the age of 42 varied from zero to a questionable grade 1. If the latter is consistently found, it suggests slight ovarian function.

The injection of adrenocorticotropic hormone for testing adrenocortical function has been employed in a number of these cases. Thorn *et al.* (7) have shown that the adrenal cortex responds in characteristic fashion in hypopituitarism caused by chromophobe tumor, if the endocrine deficiency has been of relatively short duration; but in long-standing cases there is failure of response to the test dose, just as in Addison's disease. Hence, this test on first application may not distinguish between primary (Addison's disease) and secondary (pituitary deficiency) adrenocortical failure, but larger doses at repeated intervals may evoke a fall in circulatory eosinophils as well as an increase in urinary uric acid excretion. The results can usually be corroborated on clinical grounds.

ROENTGENOLOGIC FEATURES

Roentgenographic evidence of a distorted or enlarged sella turcica associated with ballooning, thinning and depression of the floor, either bilaterally or unilaterally, is of paramount importance in diagnosis. At this clinic the size and shape of the sella are interpreted on postero-anterior and lateral roentgenograms of the skull.

The size of the sella can be measured on the roentgenogram in several ways. It can be measured roughly in two dimensions, or the surface area can be computed by tracing the outline of the sella on ruled square-millimeter paper. Recently a transparent sellameter was devised which, when placed over the sella, gives a rapid approximation of the size of the lateral contour area. A review (8) of 1,000 skull plates led us to place the arbitrary upper level of the sella area in the adult at 140 sq. mm. In our series only 2 cases were interpreted as having normal sized sellae and in these 2 cases the thinning or evidence of ballooning was present. Because of the marked visual changes, both of these patients were operated on

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four hours. Exploration revealed no adrenal tumor. A report of this case will be published in the near future.

[3] Frank method.

and found to have large extrasellar chromophobe tumors. This is an important observation and demonstrates that a normal sella alone does not exclude pituitary tumor. The area measurements in this series were mainly under 300 sq. mm., although several instances of larger sellae, ranging up to 468 sq. mm., were noted.

Thinning or absence of the posterior clinoid processes with posterior displacement and depression or breaking through of the floor into the sphenoidal sinus was frequently noted. Rarely, flecks of calcium may be observed in or above the sella in chromophobe adenoma but they are more usually seen in craniopharyngioma. After roentgen-ray therapy or surgery, recalcification both within the sella and in the clinoids is not uncommon. Reduction in size of the sella after treatment is unusual, whereas it is not unusual after treatment in acromegaly. In only a few cases was a calcified pineal body found to be displaced. Such displacement, in the absence of other causes, is regarded as evidence of a large tumor with suprasellar extension. Other roentgenologic evidence of increased intracranial pressure is rare. If there is any suspicion of suprasellar extension a pneumoencephalogram should be taken.

Roentgenologic evidence of lesions other than pituitary adenoma

In a few instances, the anterior clinoids are separated and in unilateral adenomas causing one-sided destruction of the sella, there is sharpening and elevation of the ipsilateral anterior clinoids. When this occurs, it may be indistinguishable radiologically from aneurysm of the internal carotid artery. Evidence of hypopituitarism may be present, owing to pressure from an aneurysm. The presence of calcification in the internal carotid artery in a patient with hypertension past 50 years of age should suggest aneurysm rather than chromophobe tumor. Visual field defects are unusual in aneurysm. Exploration or arteriography will establish the diagnosis. It is possible for both to be present. Aneurysm, we believe, is occasionally present in spontaneous myxedema. In these cases, the question arises as to whether the patient may have pituitary myxedema from pressure of an aneurysm, as we have seen, or whether the patient may have an aneurysm secondary to hypertension, arteriosclerosis and primary thyroid myxedema. It is in these patients that employment of the adrenal water test and other observations are helpful, as stated previously.

Enlarged sella turcica without other evidence of chromophobe adenoma

A review of the cases of enlarged sella turcica found on roentgenologic examination during the last few years revealed 44 cases in addition to the 164 which form the basis of this study. These cases were reviewed but

were not included, because of lack of evidence of chromophobe adenoma. They were found on roentgenologic examination of the skull, cervical vertebrae and nasal sinuses. Some of these may represent asymptomatic chromophobe tumors or inactive mixed tumors, in view of the findings of Costello (4).

Of the 44 cases, all but 8 were between 30 and 60 years of age. There were 18 males and 26 females. The size of the sella recorded ranged from 130 to 397 sq. mm. In all but 5 cases it was under 200 sq. mm. There were 4 cases of enlarged sella occurring in females who had been castrated for over fifteen years. Of the various tests which could be carried out to explore the endocrine status in these cases, none gave abnormal results. It is possible that asymptomatic tumors were present in these cases.

SUMMARY

A report is presented of the clinical, laboratory and roentgenologic findings in 164 cases of chromophobe adenoma of the pituitary.

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CHROMOPHOBE PITUITARY TUMORS. II. TREATMENT

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THE treatment of chromophobe pituitary tumor or its complications consists in roentgen irradiation, surgery or a combination of the two, and the administration of natural or synthetic hormonal substances when secondary glandular deficiencies exist.

In the majority of instances the primary object of treatment is the arrest or restoration of visual loss; evaluation of treatment, therefore, is based largely on its effect on this function. In general it has been thought that the secondary constitutional effects were inconstant and relatively unimportant in comparison to the danger of loss of vision, especially since little could be done to correct these effects. The restoring of vision is obviously of the greatest importance; but to achieve the best result the patient should also have relief of other symptoms, an increase in well being and an ability to return to work. The combined efforts of the neurosurgeon, radiologist and internist may be necessary to achieve this end.

ROENTGENOLOGIC TREATMENT

No definite criterion for visual acuity or field defects has guided our choice of whether the initial therapy should be irradiation or surgery. In general, especially during the past ten years, we have employed roentgen irradiation before considering operation, when the reduction in vision has not been marked or when visual loss has not been rapid. This practice is now generally favored by neurosurgeons.

The dosage of irradiation therapy for our patients has varied in amount. At present, it is customary to give a tumor dose of 4,000 roentgens by the rotational technic of Hare and Trump (1). However, the patients reported in this paper were not treated by this method. The treatment employed in this group of cases was a smaller dose, of approximately 1,800 roentgens (tumor), given through two lateral 5-cm. temporal portals.

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The improvement, if any, may be prompt or gradual, occasionally occurring many months after cessation of therapy.

There is nothing in our experience to indicate that preoperative roentgen-ray treatment has any influence on the surgical mortality, nor does it appear to increase the technical difficulties of the surgical procedure. Postoperatively, the pituitary area is irradiated routinely, giving one or two courses of such therapy. In all probability this lessens the incidence of recurrence.

If serious visual impairment exists or if visual changes have been recent and marked, a decision has to be made as to whether immediate operation or cautious irradiation should be undertaken. If the latter is carried out, the patient is carefully observed throughout the period of treatment for changes in the visual acuity and fields. Progressive deterioration of vision or visual symptoms such as scintillating scotoma are an indication to consider surgery.

Another danger to impairment of vision is hemorrhage into the adenoma occurring during roentgen-ray therapy. This may cause increased pressure to which the stretched optic nerves cannot accommodate. Symptoms of rapid visual loss or colored scotoma or both usually accompany a hemorrhage. We consider this a surgical emergency. In our series, 1 patient died during the course of roentgen-ray treatment. He lapsed into coma, with peripheral shock, and died during emergency ventriculography. Autopsy revealed, in addition to a large tumor with extension into the middle fossa, a cerebellar pressure cone as the chief cause of death.

SURGERY

Except in rare instances, all the operations for chromophobe tumor in our series have been performed by way of the intradural and transfrontal approach on the right side regardless of which eye has been most seriously affected. The main indications for surgery are:

1. Marked visual impairment.
2. Recent, rapidly advancing visual loss.
3. Progressive visual impairment despite adequate roentgen-ray treatments.
4. Visual impairment arrested at an incapacitating level, despite irradiation.
5. Large tumors with extrasellar extension causing central nervous system symptoms, sometimes requiring air injection studies to demonstrate the extent of the lesion.
6. A clinical picture not altogether typical of a pituitary tumor and compatible with another type of chiasmal lesion (Davidoff and Feiring (2)).

In the present series there were 105 patients up to December 1949,

who had been operated on for pituitary tumors a total of 133 times. In 66 instances the patients had roentgen treatment either before or after, or both before and after the surgical procedure. Of this number, 36 patients were operated on because roentgen treatment had failed to improve their vision or to maintain it at a useful level. Thirty-nine patients were subjected to operation only and had no roentgen treatment at any time.

It is obvious that from the surgical standpoint our series, like those reported by Jefferson (3), Grant (4), Davidoff and Feiring (2), and Bakay (Olivecrona's cases) (5), must be separated into different categories in order to obtain a true picture of what can be expected by operative measures, together with the dangers involved when the adenomas have invaded the intracranial cavity in various directions outside the limits of the sella turcica. Jefferson, for instance, stated that the mortality in this latter group is 33 per cent and Bakay's figure for these extensive tumors in Olivecrona's series is 35 per cent. In our group there were 29 patients who at the time of their primary operation had adenomas with marked extrasellar intracerebral invasion. There were 10 deaths among these patients, a 34.4 per cent mortality. Many had large projections upward behind the chiasm into the third ventricle or into one of the lateral ventricles, in some instances sufficient to displace the pineal shadow.

In reviewing the records of the deceased patients, many of whom were blind or with vision reduced to 20/200 or less, it was obvious that with the exception of 4 or 5 patients, neither surgery nor roentgen treatment could have been effective because of the far-reaching intracranial spread of their adenomas. It was equally obvious that a transphenoidal procedure on these tumors would have accomplished nothing. It may be argued that surgery of any kind is unwarranted in this group of patients. It is, however, impossible to foretell without exploration the exact extent of such tumors and therefore the patients were given their only chance of being helped. It is difficult to refuse operation under these circumstances, even when the chances of success are remote. Furthermore, it must be remembered that 19 of the 29 patients did survive for periods up to eighteen years, many of them with vision improved or maintained at a useful level. Again it can be said that with the survivors of this group as well as with the fatal cases it was inconceivable, because of the conditions seen at the time of the intracranial operation, that anything useful could have been accomplished by the transphenoidal procedure, which permits only a very small opening in the floor of the sella without visualization of the extent of the adenoma. It is quite possible that when an extensive adenoma is suspected, an air injection study should precede either radiation or operative therapy in order to gain more complete knowledge as

to how far and in what direction the tumor has invaded the intracranial cavity. So far, we have been reluctant to subject patients to this added procedure.

Eighteen patients of the total of 105 had multiple operations for such tumors, some of which extended backward along the base of the brain to involve the pons. In this group, obviously, many of the secondary operations were carried out as a last resort. There were 5 deaths, but it is significant that the survival periods of all the patients in this group were from two to eighteen years, with an average of seven and a half years.

Among the 76 patients not having extrasellar extension of their adenomas at the time of their primary operation there were 3 deaths, a mortality of 3.9 per cent. This, it would seem, is the really significant figure when comparing the mortality of the present-day frontal craniotomy with that of the transphenoidal procedure. Cushing's over-all mortality for both types of operations was 4.9 per cent. This exceedingly low mortality of Cushing (6), as Davidoff and Feiring pointed out, is probably due largely to the fact that radiation was seldom instituted in his cases until after operation and in all likelihood a large proportion of the tumors had no great extensions and were in a distinctly more operable stage than in many of the patients seen at the present time. Jefferson had only a 2 per cent mortality for noninvasive adenomas and Olivecrona's figure for this type of tumor was 6.4 per cent, as given by Bakay. Hirsch's (7) mortality, using only the transphenoidal procedure, was 4.7 per cent for 277 patients after primary operations between the years 1919 and 1937. This figure again doubtless reflects the fact that many of the adenomas seen in those years were in a relatively early stage.

Hyperthermia after operation is often the cause of death. At present it is our practice to administer ACTH immediately after surgery, and when hypopituitarism exists, for a few days before operation. Although our evidence as yet is not statistically significant, we believe that the use of ACTH will prove to be a valuable procedure in the reduction of surgical mortality, especially as regards the prevention or reduction of hyperthermia.

Survival periods

There were 92 patients who survived their primary operations irrespective of the size and extent of their tumors, an over-all mortality of 14.1 per cent for all types of growth with or without intracranial extension. This figure, therefore, is comparable to that of Davidoff and Feiring (13.6 per cent) and of Grant (13.1 per cent) for chromophobe adenomas. Of this number, 68 were operated on before 1945 so that a follow-up period of five years or more has elapsed. Forty-nine (72 per cent) of these

68 patients are known to have survived for from five to eighteen years, and 6 others (8.8 per cent) are known to have survived for from one to four years and may still be living, although we have no recent word from them. We have no follow-up records on 2 patients. Eleven patients (16 per cent) are known to have died within five years (from a few months up to four and one-half years) following their operations. Six of these 11 deaths were probably from tumor recurrences, whereas 5 were known to be from other causes.

Survival alone does not tell the whole story as to the value of operative treatment or a combination of surgery and radiation. Therefore, it is important to review the results from the standpoint of useful life. In the case of pituitary adenomas this revolves largely, although not wholly, around preservation or restoration of vision.

RESULTS

We have analyzed and evaluated our results in a manner similar to Henderson's (8) evaluation of Harvey Cushing's work (1939). Vision and the ability to work have been considered the chief guides in making a survey of the results of therapy. If one takes the whole group of 164 patients in this study (9), which includes the 50 cases verified only by the presence of a large sella turcica together with other pituitary stigmata, there were 141 who either survived their original operation or had radiation treatment only. One death occurred during roentgen-ray treatment. Of the survivors, 95 (67.3 per cent) may fairly be said to have been benefited by the treatment they received (radiation, surgery, or a combination of both) over a period ranging from a few weeks up to eighteen years, with an average of five and a half years. Our gauge of improvement has been based largely on improvement in visual disturbance; but in the case of radiation therapy only, improvement in other symptoms such as headaches, general physical status, or amenorrhea has been considered. In an additional 30 patients (21.3 per cent) whose vision was impaired when they came for treatment, no appreciable change in vision occurred; but it was maintained at the original level for varying periods up to eighteen years, with an average of four years. Vision became worse in 5 patients after one or the other form of treatment.

By the foregoing statements it is not meant to imply that all the patients who were either benefited or unchanged by the treatment maintained their improvement indefinitely. An appreciable number in all categories of treatment had recurrences of visual impairment, for which further treatment of one sort or another was again instituted. It has not been possible in this summary to work out these numbers in detail, hence we have given only average lengths of time during which vision was improved

or unchanged. The figures as given do not include 9 additional patients without visual defects. Five of these were treated medically with hormonal replacement and all improved. The remainder did not return for advised treatment.

Results of treatment on visual apparatus

Under this heading, only the 105 patients with histologically verified adenomas will be considered.

A comparison of roentgen irradiation and surgical treatment of chromophobe tumors is difficult because of variability in the manifestations of such tumors. There is no doubt that in some cases surgical intervention is indicated almost immediately. On the other hand, experience has demonstrated that roentgen-ray therapy will accomplish all that surgery will do in some cases. Roentgen-ray therapy is accompanied by a low mortality as compared to surgery and is therefore to be preferred; but, as pointed out before, it may fail or hemorrhage may occur, necessitating surgery. Irradiation after surgery is given to help prevent recurrence, and, judging by the available statistics, it is probable that this has been accomplished.

Surgery alone. There were 31 patients in this group whose course has been followed from three months up to eighteen years after their operations. Twenty-seven of these have been followed from two to eighteen years. In 24 instances (77.4 per cent of the total of 31) vision was improved for from three months to eighteen years—average, six years. Of these 24 patients it is known that 11 maintained their improvement for from five to eighteen years and 8 others are known to have maintained improvement for from two to four years. In 6 cases vision was unchanged for from one to five years and in 1 case vision was worse after operation.

Surgery combined with radiation. There were 60 patients in this group whose course has been followed from a few weeks up to sixteen years and of these, 46 have been followed from two to sixteen years. In 41 patients (68.3 per cent of the total of 60) vision was improved for from two to sixteen years—average, six years and two months. Of these 41 patients, the visual improvement has been maintained in 18 for from five to sixteen years, and in 16 others for periods of from two to four years. Vision was unchanged in 15 patients (25 per cent), for from a few weeks to thirteen years—average, five years and five months—and was worse in 3 patients for periods of from a few weeks to seven years. One of these patients, although blind, was at his usual occupation as a highly paid executive until his death from another cause. One patient has not been heard from.

To sum up the matter of surgery and surgery combined with radiation, there were 65 patients, or 71.4 per cent of the 91 survivors who have been heard from, whose vision was improved for periods of from a few weeks

up to eighteen years—average, six years. In 21 patients vision was unchanged for an average of four years, but in the majority of instances vision in these patients was maintained at a useful level by one or the other form of treatment. In 4 patients vision became worse and remained worse after operation or after the combination of surgery and radiation.

Surgery after failure of radiation. Among the 60 surviving patients who had a combination of surgery and roentgen-ray treatment there were 36 who were operated upon because their vision had continued to fail after radiation or had not been maintained at a useful level. Of the 32 survivors, 23 were improved after removal of their adenomas for from eight months to sixteen years—average, five and one-half years. In 8 patients vision was unchanged for from a few weeks to ten years—average, five and two-thirds years. One patient became blind after his final operation. This was the patient referred to previously who continued at his usual position as an executive for seven years. We have no follow-up for 1 patient in this group.

Results after radiation treatment only. As stated elsewhere, 50 patients in our series were treated for their presumed chromophobe adenomas only by radiation. These tumors, therefore, were "verified" by the presence of a greatly enlarged, "ballooned" sella turcica combined with either visual field defects (usually with primary optic atrophy) or other definite pituitary stigmata (amenorrhea, skin and hair changes, and so forth).

In 27 of these 50 patients radiation therapy was started because vision was sufficiently impaired to warrant it, whereas in the other 23 patients radiation was instituted because it was thought that it might benefit other symptoms which were attributed to the pituitary adenomas.

With respect to vision, 16 patients (59.3 per cent of the 27 with impaired vision) were moderately to markedly improved by roentgen-ray treatment; but in 11 (40.7 per cent of the 27) vision was either unimproved or became worse in spite of radiation.

Of the 23 patients who received radiation for other than visual impairment, vision continued to remain normal in 22, but in 1 patient vision became somewhat worse while under treatment.

Results of treatment on endocrine functions

As to recovery from hormonal deficiencies after either irradiation or surgery or both, the data show little success. It is not at all improbable that success might follow operation or even irradiation in cases of short duration. It is not likely, however, that many of these tumors will be recognized early, because of the frequent insidious onset. Early diagnosis would necessitate roentgen examination of a large number of people for a variety of symptoms which occur so commonly in other disorders. Further-

more, the sudden onset of visual impairment without other prodromal symptoms is a rather discouraging feature as regards early recognition. Certain signs and symptoms, however, should be recognized as possibly pointing to chromophobe tumor and these may be repeated here for the sake of emphasis:

Loss of libido and potentia
Amenorrhea
Myxedema
Anemia with a low basal metabolic rate
Headache
Loss of axillary, pubic and body hair
Bilateral testicular atrophy
Cessation of growth and of further development of secondary sex characteristics after 12 years of age (or even before)
Decrease in visual acuity

Occasionally, patients are encountered who have a large sella and evidences of hypopituitarism but in whom there are no visual changes. Experience has shown that roentgen-ray therapy for chromophobe tumors rarely results in improvement in the state of hypopituitarism. Not only is this true following roentgen-ray therapy but also following surgery. It is our belief, therefore, that surgery or radiation therapy for chromophobe tumor is not indicated for treatment of manifestations of hypopituitarism alone, particularly in the older age group. We have frequently raised the question as to the advisability of surgery in the young female or male in whom roentgen-ray therapy over a period of a year is not followed by improvement in gonadal function. It is possible that when one encounters a young woman with an enlarged sella and amenorrhea of short duration, surgery might bring relief and restore the gonadotropic function after roentgen-ray therapy has failed. The same might possibly be true in young males who have lost or have diminished gonadal function and diminished or absent spermatogenesis. We are confident that it is possible to restore spermatogenesis in some cases of hypopituitarism with the use of testosterone and chorionic gonadotropin. On the other hand it is extremely doubtful if ovulation and normal menstruation can be initiated by the use of estrogens or pregnancy-urine extracts in females with hypopituitarism.

Usually anemia, a low basal metabolic rate and a positive response to the water test are found in individuals who definitely have pituitary deficiency (amenorrhea excepted) from the clinical point of view. As related before, almost any combination of hormonal deficiency may be encountered in the case of chromophobe tumor; it is possible to have hot flashes with excessive FSH in the urine along with a positive response to the adrenal water test and a low basal metabolic rate. In one case men-

tioned previously (Case 5 (9)) the patient had bitemporal hemianopsia, menstruated regularly and became pregnant. Such a combination of findings brings up the possibility of mixed pituitary tumor in which there are active gonadotropic, thyrotropic or adrenocorticotropic cells.

When clinical evidence of pituitary deficiency is found, such as hypotension, anemia, loss of axillary and pubic hair, amenorrhea, low basal metabolic rate, and positive response to the water test, our usual plan of therapy is to administer desoxycorticosterone along with testosterone by pellet implantation to both males and females. After this has been carried out for a month or two, desiccated thyroid is tried. This method may reduce the possibility of producing an adrenal crisis by the administration of desiccated thyroid, a rare but real danger.

During the past two years ACTH has been administered to 12 patients (not all from this series). Its effects have been exceedingly gratifying in that greater endurance and a feeling of well-being have resulted. It is our impression that it is still worth while to administer testosterone and desoxycorticosterone, as the daily requirement of ACTH may be lessened. The maintenance dose of ACTH has varied from 10 mg. every other day to 25 mg. a day. More recently, because of sensitivity to ACTH or for other reasons, cortisone has been used orally instead. A satisfactory daily dose of cortisone appears to be 25 mg., although as little as 15 mg. daily has sufficed in cases with marked hypopituitarism. When cortisone is discontinued, no serious complications arise; there is merely a gradual return to the previous state.

In patients manifesting clinical evidence of myxedema, definite and often marked improvement is obtained with desiccated thyroid alone, whereas such therapy may not help patients with panhypopituitarism who do not manifest such clinical evidence. Desoxycorticosterone causes an increase in blood pressure and we believe an increase in the sense of well-being. There is some doubt that desoxycorticosterone affects only salt metabolism. Observations on Addison's disease indicate that it may also affect other metabolic processes, causing general improvement not entirely due to the changes in salt metabolism, though such improvement may be due to improved nutrition. Testosterone causes retention of nitrogen and synthesis of protein and apparently lessens ease of fatigue. It also causes regrowth of pubic, axillary and body hair which, although of no practical importance, probably has a psychologic value. There is little to fear as regards masculinizing effects with testosterone in females with hypopituitarism.

Much to our surprise, we found in several cases that the continuation of desoxycorticosterone is not always necessary in hypopituitarism, since the blood pressure may remain elevated long after the pellets are exhausted. The use of estrogens has not been found particularly worth

while, except to correct uncomfortable atrophic vaginal changes and to produce periodic vaginal bleeding.

In males, libido may be restored and nocturnal emissions may occasionally follow the use of adequate doses of testosterone. This may even occur in patients past 50 or 60 years of age. Spermatogenesis (10) was observed after treatment with chorionic gonadotropin and testosterone in 1 case of panhypopituitarism without an enlarged sella and presumably not associated with a pituitary tumor.

Spontaneous improvement in the red blood cell count and hemoglobin

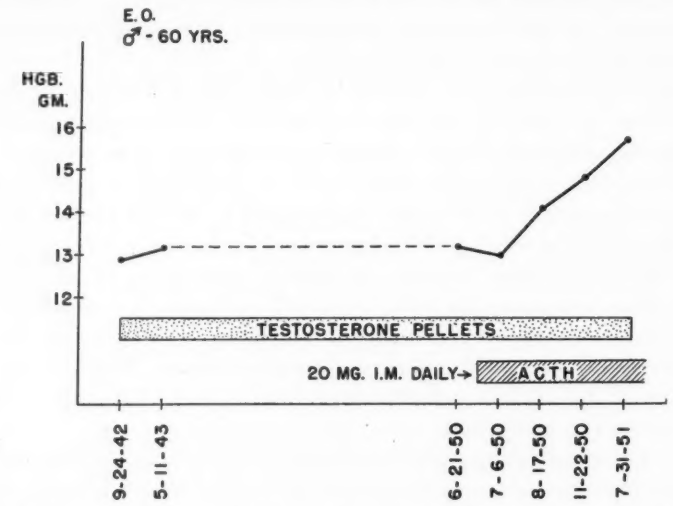

FIG. 1. Chromophobe tumor and hypopituitarism in a man, aged 60; duration twenty years. Testosterone pellets were implanted every three months for eight years up to the present time. ACTH, 20 mg. daily, has increased hemoglobin and erythrocytes.

is most unusual. Iron and liver have been ineffective. Under combined treatment with desoxycorticosterone, testosterone and desiccated thyroid, an increase has been noted in hemoglobin and red cell count (rarely ever to normal levels) which may be the result of testosterone alone. ACTH appears to have been more effective in this regard (Fig. 1). In several cases in which cortisone has been used, no change in hemoglobin has yet taken place. In patients who manifest clinical evidence of myxedema, administration of desiccated thyroid may account for some increase in hemoglobin and red blood cell count.

SUMMARY

A report is presented of the results of treatment by surgery, roentgen

rays, or a combination of both, in a series of cases of chromophobe adenoma of the pituitary, of which 105 were verified histologically.

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THE DOSE RESPONSE IN MAN TO ORAL AND INJECTED LIPO-ADRENAL CORTICAL EXTRACT

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THE oral administration of adrenal cortical extract (ACE) was reported by Osler in a case of Addison's disease over fifty years ago (1). A comparison of the effectiveness in Addison's disease of a glycerol extract of adrenals, given orally and injected as reported by Thorn *et al.* (2), indicated that two and a half times the parenteral amount of hormone was needed orally for equal effect. However, Conn and Silverman (3) showed that the oral requirement was one half of that needed for injection. In both of these publications there were reported physiologic changes such as the increase in potassium output and the retention of sodium, attesting to the effectiveness of the orally administered hormone.

Metabolic changes also have been reported by Freeman and his colleagues at the Worcester State Hospital (4, 5) when extract of the adrenal cortex was given orally to schizophrenic patients. Decrease in lymphocytes was observed, together with alteration in the urine output as well as changes in the specific gravity of the urine and a decrease in the cholesterol of the serum. Increase in the blood pressure was a consistent finding with this extract.

Noble (6) reviewed a number of researches with animals, especially rats, giving evidence that the aqueous extract (ACE) was just as effective when given to adrenalectomized animals in water or with food as when it was injected. Some reports claimed greater activity with oral administration. Data presented by Kuizenga *et al.* (7) demonstrated that corticosterone, dehydrocorticosterone and ACE were just as effective when given orally as when injected, but 11-desoxycorticosterone acetate proved to be thirty-five times more effective when injected. All of these animal experiments were based on the survival and growth of immature adrenalectomized rats. Activity orally was reported by Elmadjian and Pincus (8) in a stress test with lipo-adrenal cortical extract (LAE) and corticosterone. This paper contains dosage-response data on oral and injected LAE (Upjohn) in man.

METHODS

Lipo-adrenal cortical extract (LAE) was given to human subjects (male schizophrenics and normal young men) in doses of 20 cc., 10 cc., 5 cc., and 2.5 cc. (1 cc. extract≈2 mg. cortisone), intramuscularly and

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TABLE 1. THE MEAN PERCENTAGE CHANGES IN VARIOUS URINE AND BLOOD DETERMINATIONS AFTER

Dosage of LAE and diagnosis	URINE																	
	Urine volume				*17-Ketosteroids*				*Creatinine*				*Uric acid*					
	I.M.		Oral		I.M.		Oral		I.M.		Oral		I.M.		Oral		I.	
	(1)*	(2)*	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	
40 mg.,† Schizophrenic male	5.35‡ ±1.04 3	2.80 ± .605 3	3.85 ± .390 3	2.48 ± .369 3	1.66 ± .210 3	1.07 ± .056 3	2.56 ± .948 3	1.42 ± .476 3	1.45 ± .324 3	1.07 ± .157 3	1.34 ± .056 3	1.23 ± .159 3	1.93 ± .285 2	1.68 ± .025 2	1.38 ± .659 2	1.28 ± .350 2	1.87 ± .130 2	
20 mg., Schizophrenic male	4.12 ± .768 6	2.61 ± .387 6			1.45 ± .426 6	1.26 ± .264 6			1.22 ± .084 6	1.17 ± .055 6			1.19 ± .104 6	1.15 ± .138 6			1.50 ± .187 6	
20 mg., Normal young male			3.08 ± .459 11	1.76 ± .350 11			1.43 ± .225 11	1.31 ± .177 11			1.23 ± .225 11	1.10 ± .103 11			1.40 ± .121 11	1.08 ± .121 11		
10 mg., Schizophrenic male	2.31 ± .249 3	1.61 ± .452 3	4.15 ±2.056 4	3.03 ±1.246 4	1.96 ± .410 3	1.67 ± .801 3	4.21 ±1.783 4	2.59 ± .379 4	1.16 ± .079 3	1.18 ± .197 3	1.43 ± .341 4	1.56 ± .156 4	1.82 ± .574 3	1.64 ± .590 3	1.45 ± .248 4	1.73 ± .129 4	1.72 ± .442 3	
5 mg., Schizophrenic male	1.32 ± .244 4		1.19 ± .259 4		missing		missing		1.13 ± .056 4		1.01 ± .081 4		1.23 ± .150 4		1.15 ± .066 4		1.4 ± .1 4	
Control: Schizophrenic male			1.93 ± .138 3	2.15 ± .742 3			.73 ± 1.92 3	1.51 ± .511 3			.88 ± .076 3	.82 ± .039 3			.80 ± .100 3	.72 ± .032 3		

* (1) and (2) indicate urine collections after LAE (*see* text).
** (1), (3) and (4) indicate number of hours after administration of LAE.
† 1 cc. of extract is equivalent to 2 mg. of cortisone.
‡ 1.00 = 100%.

$$\text{Standard error} = \pm \sqrt{\frac{\Sigma d^2}{n(n-1)}}.$$

Numeral after each value represents the number of determinations made.

FTER ORAL AND INJECTED LIPO-ADRENAL CORTICAL EXTRACT. (THE CONTROL VALUE IS TAKEN AS 100%.)

								BLOOD									
Potassium				*Sodium*				*Lymphocytes*						*Eosinophils*			
I.M.		Oral		I.M.		Oral		I.M.			Oral			I.M.			Oral		
(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)**	(3)**	(4)**	(1)	(3)	(4)	(1)	(3)	(4)	(1)	(3)	(4)
1.87 ± .130 2	2.58 ± .790 2	.97 ± .315 2	1.24 ± .230 2	.99 ± .247 3	.32 ± .061 3	.97 ± .247 3	.95 ± .139 3	.73 ± .101 3	.66 ± .066 3	.89 ± .094 3	1.02 ± .201 3	.88 ± .055 3	1.04 ± .347 3	.75 ± .037 3	.37 ± .057 3	.27 ± .037 3	.81 ± .155 3	.59 ± .100 3	.67 ± .103 3
1.50 ± .187 6	1.46 ± .331 6			1.38 ± .285 5	.42 ± .041 6				.82 ± .056 6	.82 ± .053 6		.93 .120 3	.88 .092 3						
		1.59 ± .153 11	1.04 ± .154 10			1.59 ± .201 10	1.20 ± .173 10				1.12 ± .095 5	.98 ± .063 12	.92 ± .052 12						
.72 ± .442 3	1.75 ± .460 3	1.51 ± .457 4	1.33 ± .197 4	1.63 ± .324 3	.76 ± .438 3	1.52 ± .421 4	1.43 ± .116 4	1.07 ± .209 3	.99 ± .101 3	1.01 ± .173 3	.87 ± .072 9	.80 ± .050 9	1.34 ± .174 9	.68 ± .101 3	.57 ± .062 3	.59 ± .059 3	.79 ± .111 9	.71 ± .152 9	.70 ± .113 9
1.42 ± .155 4		0.94 ± .218 4		0.96 ± .158 4		1.23 ± .242 4		.86 ± .103 4	.72 ± .069 4	.99 ± .142 4	1.14 ± .158 4	.94 ± .127 4	1.25 ± .129 4	1.21 ± .094 3	1.05 ± .028 3	1.05 ± .133 3	.95 ± .044 3	.97 ± .050 3	1.03 ± .032 3
		1.27 ± .469 3	.57 ± .110 3			1.11 ± .217 3	.98 ± .172 3												

orally. Determinations were made of the output of urine, creatinine, 17-ketosteroids, uric acid, potassium and sodium, and of the changes in the blood lymphocytes and eosinophils. The control sample was taken during the two hours after waking in the morning from approximately 6:30 to 8:30 A.M. The hormone was given at 8:30 A.M. Following medication, urine was collected in two separate samples covering: 1) the period from 8:30 A.M. to 10:30 A.M., and 2) the period from 10:30 A.M. to approximately 1:00 P.M. The water intake was standardized by the ingestion of a glass of water at 6:30, 8:30 and 10:30 A.M. The subjects were fasted. Blood samples were obtained just before the hormone administration (designated as 0 hour) and at 1, 2, 3 and 4 hours following. Methods employed for the various determinations were the same as in a previous study (9).

Results are expressed in percentage of the control (premedication) sample (Table 1). With regard to Figure 1, the urine determinations represent the average of the two post-medication samples. The blood counts are given as a percentage of the 0-hour sample. Lymphocytopenia is maximal at three hours after LAE administration and eosinopenia at four hours.

DISCUSSION

Considering the over-all adrenal cortical response noted in these dosage ranges, the effectiveness of the orally administered extract proved to be comparable to that of the injected hormone (Fig. 2). The total response index (TRI) (9) is composed of the average percentage changes of the adrenal indices, with the exception of urine volume and sodium.

With respect to the individual indices, we may gather from the data that increases in adrenal cortical activity were discernible, with uric acid and 17-ketosteroids as the index (Table 1). The values, however, show no clear quantitative relation to dosage. Urine volume and eosinophil changes proved to be more proportional to the dosage than did potassium, sodium or lymphocytes (Figs. 1 and 2). The dosage level of 5 cc. of extract (equivalent to 10 mg. of cortisone) was the minimum which was effective in either the oral or the injected series, using the eosinophil count as the response index. This minimal effective dosage level is also evident from the inspection of the sodium and potassium values.

Urinary volume excretion in both the oral and the injected series proved to be proportional to hormone dosage in this experiment. This aspect has not been investigated quantitatively before in humans, though many suggestive indications are present in the literature (10).

The potassium excretion showed a pattern different from that of the urine volume excretion (Fig. 1). As may be noted, there was a quantitative

response to the injected hormone; but with oral administration the potassium excretion levelled off to a plateau at the 20-mg. equivalent of cortisone (10 cc. of extract). With regard to the sodium excretion, a qualitative difference was evident when the oral and parenteral routes were compared. At the lower oral dosage levels there was an increase of sodium excretion, but there was a return to pre-medication levels with the higher dosages.

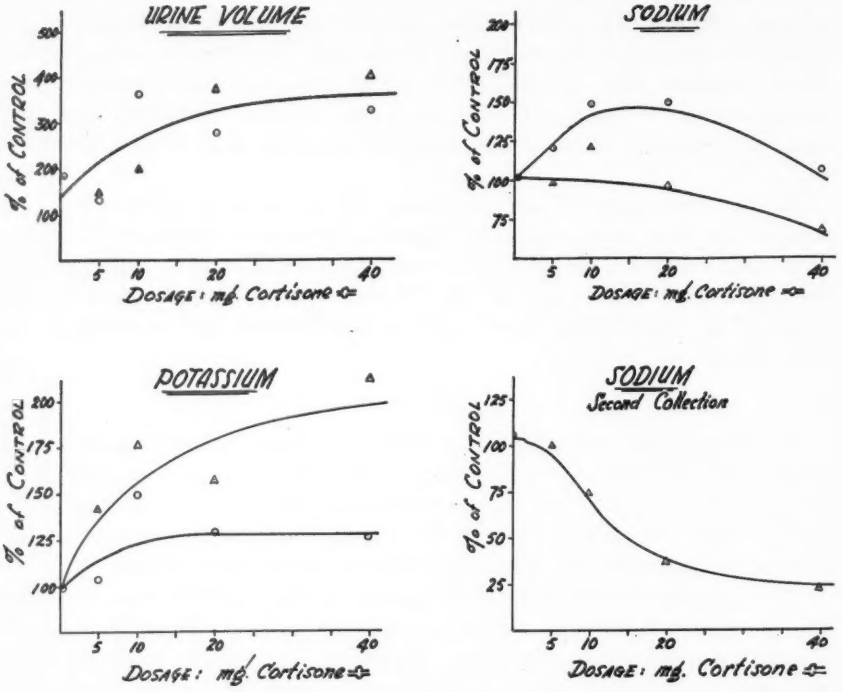

FIG. 1. *Dosage response values after lipo-adrenal cortical extract in humans.* Urine determinations represent the average of the two post-medication samples. △ = intramuscular. ⊙ = oral.

In the case of the injected hormone, there was a fairly clear retention of sodium with increased dosage (Fig. 1). The sodium retention was more striking when the values for the second period of urine collection (10:30 A.M. to approximately 1:00 P.M.) were plotted against dosage. The possibility may be considered that passage through the liver of the orally ingested material leads to the greater destruction of a sodium-retaining factor.

SUMMARY

Lipo-adrenal cortical extract (LAE) has been given to human subjects

in doses of 20 cc., 10 cc., 5 cc., and 2.5 cc., intramuscularly as well as orally.

Changes from pre-medication levels were noted in the volume of urine, in the excretion of creatinine, 17-ketosteroids, uric acid, potassium and sodium, and in the blood eosinophil and lymphocyte counts, during a four- to five-hour period following the administration of the extract.

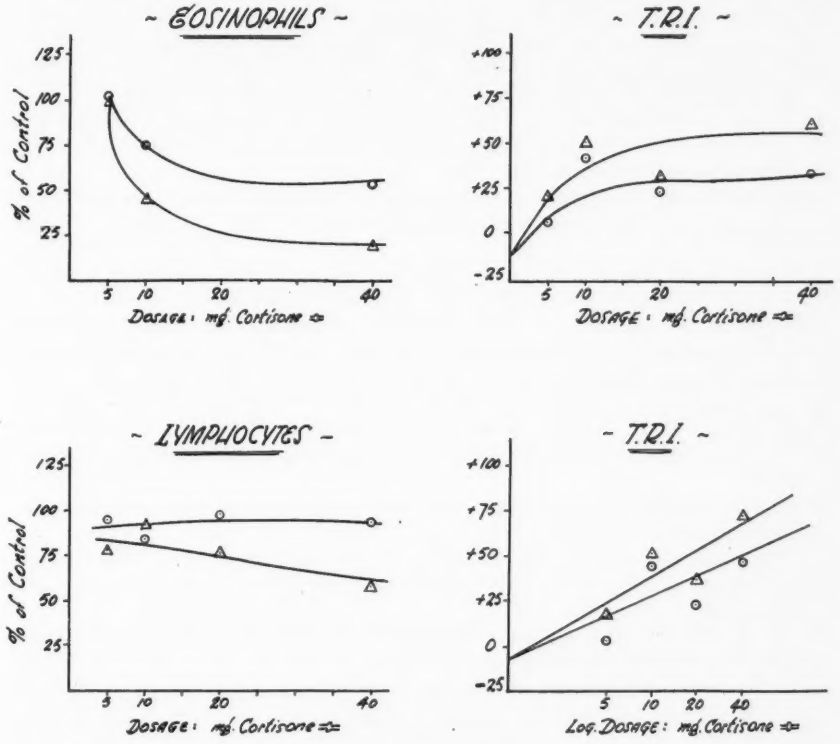

FIG. 2. *Dosage response values after lipo-adrenal cortical extract in humans.* Blood counts are given as a percentage of the 0-hour sample. Lymphocytopenia is maximal at three hours and eosinopenia at four hours. △ = intramuscular. ⊙ = oral. TRI = Total response index, which is composed of the average percentage changes of the adrenal indices, with the exception of urine volume and sodium.

Considering the over-all total response index (TRI), the orally administered extract was comparable to the injected.

Urine volume and blood eosinophil changes were quantitatively most reflective of dosage change.

The only qualitative difference observed between the effects of oral and

of injected LAE was in the sodium excretion. No sodium retention occurred even at high oral dosage levels, whereas sodium retention was induced by the parenteral doses used.

Acknowledgment

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QUALITATIVE STUDIES OF NEUTRAL 17-KETOSTEROIDS IN NORMAL SUBJECTS*†

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TOTAL urinary neutral 17-ketosteroids have been studied extensively as an index of human adrenal cortical activity, although their exact relationship with this activity is not known (1). The urinary 17-ketosteroids may be conversion products of other steroid substances. Administration of any of a wide variety of C_{19} steroids increases the output of urinary 17-ketosteroids, especially androsterone and etiocholanolone (2, 3). Mason and Kepler (4, 5), and Sayers *et al.* (6) have suggested that urinary 17-ketosteroids are breakdown products of 21-carbon-atom adrenal cortical steroids. Conn (7) and Polley and Mason (8) reported an increased urinary excretion of 17-ketosteroids following administration of adrenal cortical hormones. Sprague *et al.* (9) indicate that cortisone may depress urinary 17-ketosteroid excretion due to depression of some of the functions of the cortices, though an increase is reported in patients suffering with Addison's disease. Homburger (10) and Cuyler *et al.* (11) report no increase in urinary 17-ketosteroids on administration of adrenal cortical hormones.

These observations have led to the concept that the 17-ketosteroids or related C_{19} steroids are elaborated as such by the adrenal cortex and further, that they become altered by participation in metabolic activities and finally are excreted in a variety of forms in the urine (12–15).

Numerous workers have demonstrated by chromatographic fractionation techniques the variety of 17-ketosteroids in the urine and variations from the normal pattern in disease conditions (16–20).

This paper describes a chromatographic method for studying hourly 17-ketosteroid excretion in normal subjects, demonstrating individual variations under differing physiologic states.

METHODS

Three subjects, 1 female and 2 male, were chosen for study. Subject D was a 25-year-old, married, female medical student, 167 cm. tall and

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† Purified 17-ketosteroids used in these studies were provided by Ciba Pharmaceutical Products, Summit, N. J., and Syntex, S. A., Mexico, D. F.

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weighing 61 Kg. Subject C, a 35-year-old physiologist, was 184 cm. tall and weighed 73 Kg. Subject W was a 28-year-old physician, 182 cm. in height and 73 Kg. in weight. All subjects were considered healthy.

The psychologic nature of each individual in relation to the exigencies of everyday living was studied by means of subjective diaries recording activities and feeling states. During the periods of observation, no attempt was made to regularize or control the daily activities of the subjects.

Eosinophil counts as an index of circulating 11-oxycorticosteroids were determined in subjects C and W, according to the method described by Forsham *et al.* (21).

The method of Drekter *et al.* (22) was used to determine the total neutral 17-ketosteroids in 5-cc. portions of urine. All 17-ketosteroid determinations are given in milligram equivalents of dehydroisoandrosterone acetate. When the 17-ketosteroid content of the urine specimens was to be analyzed chromatographically, the whole specimens were hydrolyzed by adding 30 cc. of concentrated hydrochloric acid per 100 cc. of urine and heating to 80° C. for ten minutes (Drekter (22)). The hydrolyzed specimen was extracted with a quantity of absolute ethyl ether approximately four times its volume. The ether extract was washed with 10 per cent sodium hydroxide to remove the phenolic estrogens and other chromogenic materials. After a final wash with distilled water, the ethereal extract of neutral 17-ketosteroids was transferred to a flask and the ether removed under reduced pressure. The dried extract was stored in the refrigerator until analyzed chromatographically. Most of the urine specimens were processed within twelve hours after being collected, although some specimens were acidified with hydrochloric acid and stored in a refrigerator for as long as seventy-two hours.

Inasmuch as it was planned to study the 17-ketosteroid variations occurring over short periods of time, the choice of the method for chromatographic separation of these substances depended upon its applicability to that amount of 17-ketosteroid material excreted during the period of one hour. None of the methods reported prior to the beginning of these studies was found suitable for this purpose. This necessitated the development of a new method. Experimentation with a variety of specimens of alumina and elution techniques led to a satisfactory method. The technique of Dingemanse *et al.* (17) was a helpful guide. The alumina selected was that produced by Merck and Co., Rahway, N. J., and labelled "suitable for chromatographic adsorption." The activity of this alumina, tested by the method of Brockmann and Schodder (23) employing specific test dyes, was Grade II in a classification of five grades of activity. Samples from different bottles demonstrated closely similar degrees of activity, and no

alteration in the activity of the alumina was noted if the reservoir bottles were kept tightly stoppered.

The alumina columns for the adsorption of the urinary extracts were prepared in a cylindrical glass tube approximately 15 cm. long with a constant inner bore diameter of 0.9 cm. and a sintered glass sieve at the lower end. A 600-cc. reservoir with a built-in, uniform-drop pipette tip was attached to the top of the tube by a ground-glass joint. For the preparation of the column, the tube was filled with benzene, and 6.5 Gm. of alumina was introduced from above as a slurry and allowed to settle by gravity to form a column approximately 10 cm. in height. A disc of filter paper 0.9 cm. in diameter was placed on top of the column to prevent any disturbance of the alumina particles after elution was begun. A small amount of solvent was allowed to stand above the column of alumina at all times to insure constant solvent-alumina relationships. The columns of alumina thus prepared were stoppered and allowed to stand overnight. Before the start of the chromatogram, 100 cc. of benzene was washed through the column. The material to be chromatographed was taken up in 25 cc. of thiophene-free benzene and quantitatively transferred to the top of the column for adsorption. The chromatogram was then developed by the passage of the following solvents through the column:

100 cc. benzene
675 cc. benzene with 0.1% ethanol
250 cc. benzene with 0.5% ethanol
50 cc. ethanol.

Thus, a total of 44 fractions of 25 cc. each was collected. Air pressure was applied to the top of the column to insure a rate of flow of approximately 25 cc. of solvent every six to eight minutes. A 10-cc. aliquot of each sample was measured, the solvent evaporated in a water bath, and the 17-ketosteroid content of the fraction determined by the Zimmermann reaction.

It was noted during preliminary experiments that the heavier degrees of exercise were frequently accompanied by low outputs of urine. To overcome this difficulty, a measured amount (1,000 cc.) of water was drunk before each period of exercise. To determine whether the increased fluid intake and the accompanying diuresis caused changes in the 17-ketosteroid excretion, experiments, including water drinking without exercise, were also performed. In all cases, urine specimens were collected during three consecutive periods of approximately one hour each, from 9:00 A.M. until noon. On days when the effect of increased fluid intake was studied, water (500 cc.) was drunk at the beginning of the first and of the second hours. Exercising on the motor-driven treadmill with 15 per cent grade was accomplished during the second hour. Routine total 17-ketosteroid determinations were made on a sample of each urine specimen, the balance being submitted to chromatographic fractionation. Corrections were made for slight discrepancies in the times of urine collection and for aliquots removed, so that all results might be reported in milligrams excreted per hour.

RESULTS

Observations on the chromatographic method. Figures 1, 2 and 3 illustrate examples of chromatographs obtained. The graphs were prepared by plotting 17-ketosteroid values along the ordinates and the consecutive fractions collected along the abscissas. The separate chromatographing of

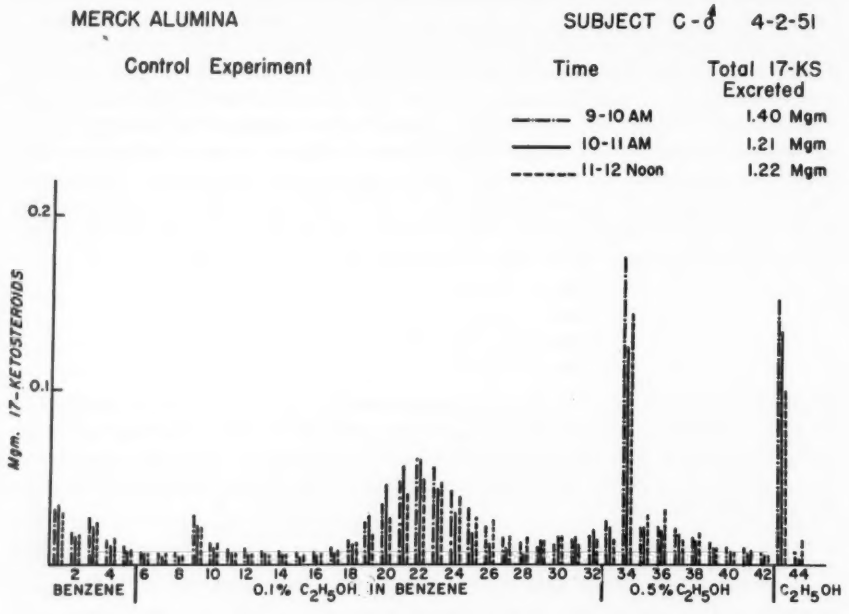

FIG. 1. 17-Ketosteroid patterns resulting from the chromatographic fractionation on Merck alumina of extracts of urine excreted during three consecutive hours. (Subject C.)

identical specimens showed an excellent reproducibility of the 17-ketosteroid pattern (Fig. 4). Five distinct peaks occurred in the chromatograms of 2 subjects (C and D) and six peaks in that of the third (W). An attempt was made, by indirect methods, to identify tentatively the 17-ketosteroid contents of the various peaks. Solutions of a number of known 17-ketosteroids were chromatographed singly and in combination with urine extracts. The added substances were recovered quantitatively and appeared in the same area in the chromatogram, whether chromatographed separately or in mixtures with other 17-ketosteroids. There was no evidence of isomerism occurring on the alumina column. The 17-ketosteroids added and the fractions in which they appeared are listed in Table 1.

TABLE 1. POSITION IN CHROMATOGRAMS OF CERTAIN 17-KETOSTEROIDS

Known 17-ketosteroids chromatographed	Fractions in which eluted
1. Androstanone-17	1–2
2. Dehydroisoandrosterone acetate	3–6
3. Etiocholanedione-3,17	9–10
4. Androstanedione-3,17	9–10
5. Δ^4-Androstenedione-3,17	10–12
6. Dehydroisoandrosterone	16–25
7. Isoandrosterone	16–25
8. Androsterone	20–29

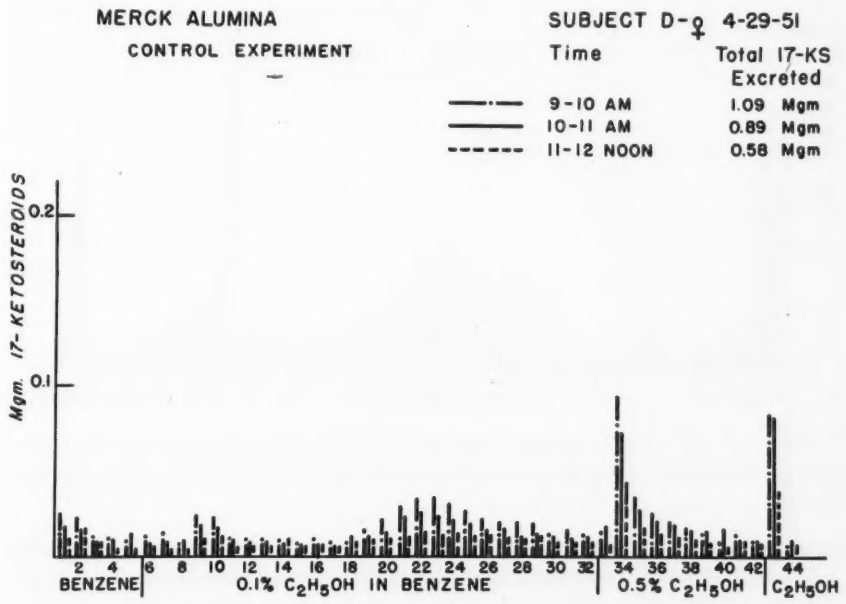

FIG. 2. 17-Ketosteroid patterns resulting from the chromatographic fractionation on Merck alumina of extracts of urine excreted during three consecutive hours. (Subject D.)

A survey of the ultraviolet absorption of the various peaks revealed a high degree of absorption with a maximum at 240 mμ in fraction number 1 of subject W. This indicates the presence of a significant quantity of $\Delta^{3,5}$-androstadienone-17 (24).

Samples of etiocholanolone and the 11-oxygenated 17-ketosteroids were

not available for study. However, as pointed out by Lieberman *et al.* (24), the order of elution of the various 17-ketosteroids from alumina is constant. Consequently, by comparing our chromatograms with those of Lieberman *et al.* (24), Dingemanse *et al.* (17), and Robinson and Goulden (19), we are able to surmise that fractions 34 to 38 contain etiocholanolone and fraction 43 contains the 11-oxygenated 17-ketosteroids plus a number

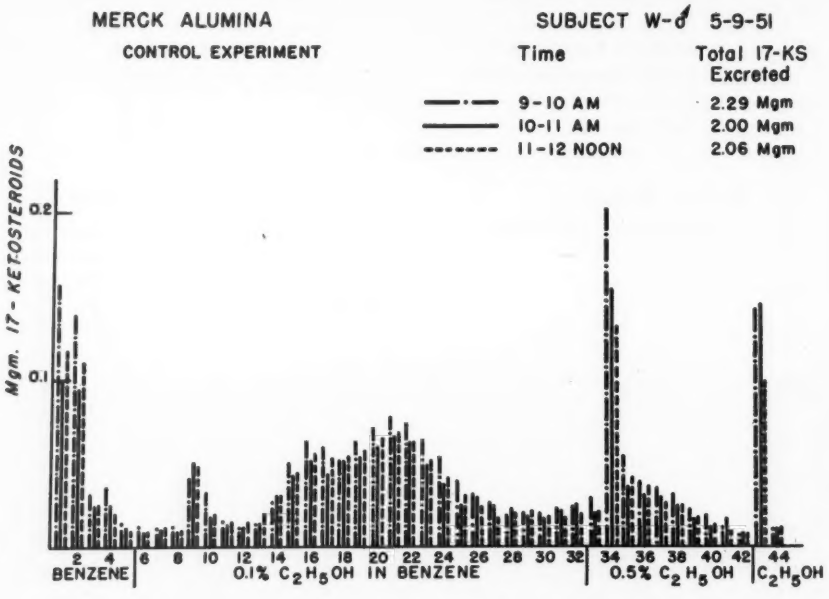

FIG. 3. 17-Ketosteroid patterns resulting from the chromatographic fractionation on Merck alumina of extracts of urine excreted during three consecutive hours. (Subject W.)

of artefacts. None of the above findings or observations is more than suggestive evidence of the contents of the various peaks noted in the chromatograms.

Although peaks occurring in fractions 15 to 29 occasionally shifted to the right or left, it was not a problem. This shifting appeared to result more from the degree of tightness with which the column of alumina was packed, as indexed by the amount of pressure required to maintain the prescribed rate of flow, than from the qualitative or quantitative content of 17-ketosteroid material adsorbed on the alumina. The failure of significant alterations to appear in the patterns obtained when relatively large or relatively small amounts of 17-ketosteroid were chromatographed was attributed to an excess of alumina in the column.

Circulating blood eosinophils. Fluctuations in the levels of the circulating eosinophils were studied in 2 of the 3 subjects. Subject C showed an average morning eosinophil count of 240/cu. mm. On control days the levels had dropped 40 per cent by mid-day, and by late afternoon had risen to 8 per cent below the initial level. On days of exercise, the average morning levels were 12 per cent, the mid-day levels 45 per cent and the late afternoon levels 20 per cent below average control morning levels.

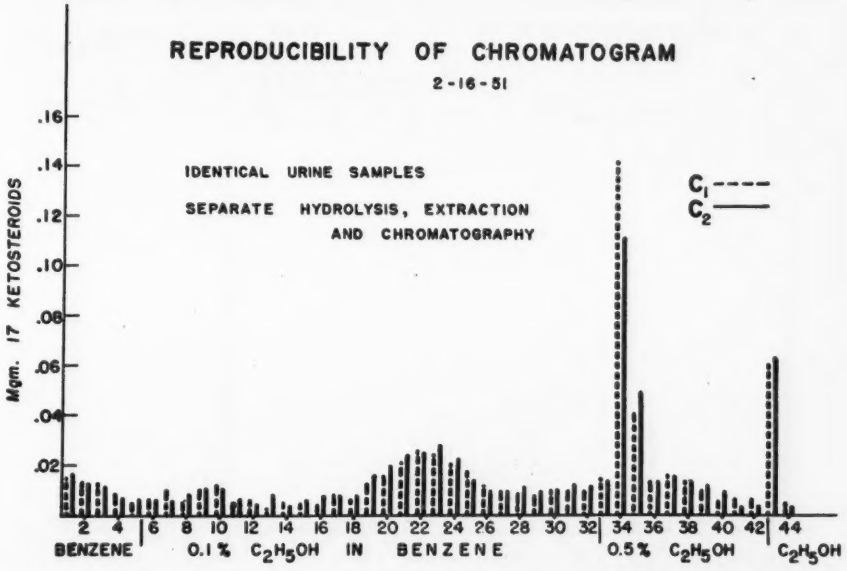

FIG. 4. 17-Ketosteroid patterns resulting from the separate hydrolysis, extraction and chromatographic fractionation of identical 1-hour urine specimens, demonstrating the reproducibility of the method. (Subject C.)

Subject W showed average morning eosinophil levels of 34 per cu. mm. On control days, mid-day levels had dropped an average of 8 per cent, and late afternoon levels had risen to 22 per cent above the initial levels. On days when exercise was performed, morning levels were 35 per cent below the control, mid-day levels were 56 per cent below the morning control, and late afternoon levels were 3 per cent below the morning control level.

Chromatographic studies of urinary 17-ketosteroids applied to subjects. Figures 1–3 and 5–8 show typical chromatograms obtained on the 3 subjects. The chromatograms of the 3 subjects differ both qualitatively and quantitatively. On days when exercise was not performed, the chromatographic totals of the hourly excretion of the 3 subjects usually diminished

progressively. These diminutions were in no way limited to a single fraction. Certain fractions persisting without essential change during the three-hour period on some days significantly diminished with time on other days. This is clearly demonstrated in the fused peaks occurring in fractions 16 to 29 of the chromatograms of subject W. It was further noted that occasionally certain peaks increased slightly, although the total

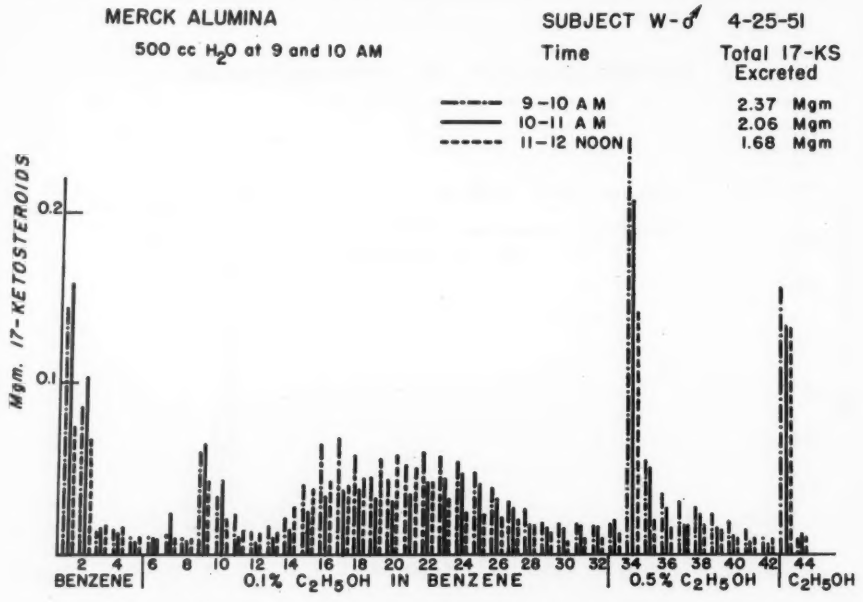
MERCK ALUMINA
500 cc H_2O at 9 and 10 AM
SUBJECT W-♂ 4-25-51
Time
Total 17-KS Excreted
9-10 A M 2.37 Mgm
10-11 A M 2.06 Mgm
11-12 NOON 1.68 Mgm
Mgm. 17-KETOSTEROIDS
0.2
0.1
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44
BENZENE
0.1% C_2H_5OH IN BENZENE
0.5% C_2H_5OH
C_2H_5OH

FIG. 5. 17-Ketosteroid patterns resulting from the chromatographic fractionation on Merck alumina of extracts of urine excreted during three consecutive hours. Fluid was forced at the beginning of the first and second hours. (Subject W.)

17-ketosteroid content of the chromatogram decreased. Comparison of chromatograms obtained on days when no extra water was drunk with those obtained on days when fluid was forced reveals no consistent qualitative difference. It was noted, however, that in subjects C and W the average total hourly excretion on the days when excess water was taken was slightly higher than on the days with normal fluid intake (Table 2).

Examples of chromatograms obtained on days when exercise was performed are presented in Figures 6, 7 and 8. During exercise subject C showed a drop in 17-ketosteroid output of 36 to 41 per cent of the previous hour's output. The average drop in 17-ketosteroid output from the first to the second hour noted on days without exercise was 12 per cent, the greatest drop noted being 14 per cent. The drop in 17-ketosteroid output

TABLE 2. COMPARISON OF 17-KETOSTEROID TOTALS DETERMINED AFTER CHROMATOGRAPHING ON CONTROL DAYS AND ON DAYS OF DIURESIS

Subjects	Days without extra water	Days with extra water
C	1.17 mg./hr.	1.23 mg./hr.
D	0.85 mg./hr.	0.85 mg./hr.
W	1.84 mg./hr.	1.93 mg./hr.

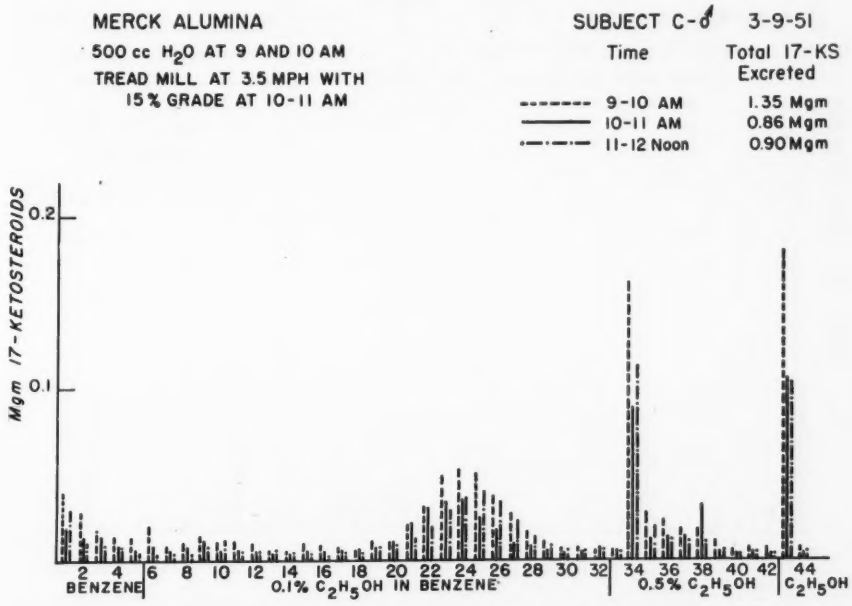
MERCK ALUMINA
500 cc H_2O AT 9 AND 10 AM
TREAD MILL AT 3.5 MPH WITH 15% GRADE AT 10-11 AM
SUBJECT C-♂ 3-9-51
Time
Total 17-KS Excreted
9-10 AM 1.35 Mgm
10-11 AM 0.86 Mgm
11-12 Noon 0.90 Mgm
Mgm 17-KETOSTEROIDS
0.2
0.1
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44
BENZENE
0.1% C_2H_5OH IN BENZENE
0.5% C_2H_5OH
C_2H_5OH

FIG. 6. 17-Ketosteroid patterns resulting from the chromatographic fractionation on Merck alumina of extracts of urine excreted during three consecutive hours. Fluid was forced at the beginning of the first and second hours and heavy exercise was performed on the treadmill during the second hour. (Subject C.)

during exercise was equally distributed among all the peaks, and no essential difference was noted between the day of light exercise and the days of heavy exercise. On the day of light exercise and on one day of heavy exercise, the level of 17-ketosteroid output during the post-exercise hour in relation to the output during the pre-exercise hour was equal to or slightly greater than that on non-exercise days. During the second heavy exercise experiment, however, the post-exercise 17-ketosteroid output remained significantly decreased in relation to the pre-exercise hour (Table 3).

Subject W showed no significant decrease in 17-ketosteroid output

with light exercise. With heavy exercise, however, this 17-ketosteroid output decreased 52 to 81 per cent from the pre-exercise hour to the exercise hour. The average decrease in 17-ketosteroid output between these hours on days without exercise was 15 per cent, with the greatest drop being 32 per cent (Table 3). As in the case of subject C, the drop in the 17-ketosteroid excretion was distributed equally among all the ketosteroids. On both days of heavy exercise, the post-exercise excretion of

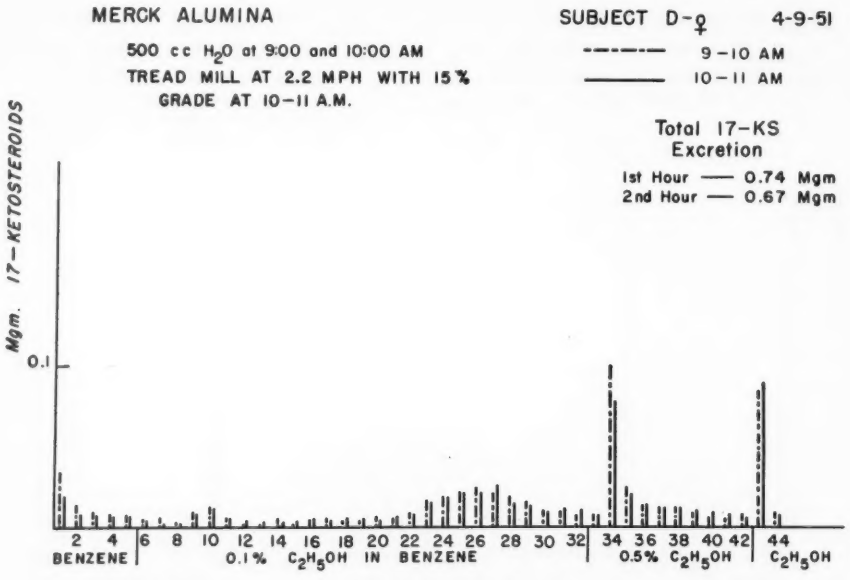
MERCK ALUMINA
500 cc H_2O at 9:00 and 10:00 AM
TREAD MILL AT 2.2 MPH WITH 15% GRADE AT 10–11 A.M.
SUBJECT D-♀ 4-9-51
9–10 AM
10–11 AM
Total 17-KS Excretion
1st Hour — 0.74 Mgm
2nd Hour — 0.67 Mgm
Mgm. 17-KETOSTEROIDS
0.1
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44
BENZENE
0.1% C_2H_5OH IN BENZENE
0.5% C_2H_5OH
C_2H_5OH

FIG. 7. 17-Ketosteroid patterns resulting from the chromatographic fractionation on Merck alumina of extracts of urine excreted during two consecutive hours. Fluid was forced at the beginning of both hours, and light exercise was performed on the treadmill during the second hour. (Subject D.)

17-ketosteroids in relation to the pre-exercise hour was equal to or slightly greater than that on non-exercise days.

In considering the drop in the excretion of 17-ketosteroids occurring with heavy exercise, a decrease in the output of urine from the pre-exercise to the exercise hour (Table 3) was invariably noted. Further observations were made to determine whether the drop in 17-ketosteroids and the drop in urine output were correlated. A total of 133 routine 17-ketosteroid determinations in terms of hourly excretion were plotted against the volume of urine excreted during the same period. A positive r of 0.26 was noted. Significant amounts of 17-ketosteroids frequently occurred

TABLE 3. COMPARISON OF HOURLY URINE OUTPUT AND HOURLY 17-KETOSTEROID EXCRETION

	Hour of collection	Subject C		Subject W		Subject D	
		cc. urine /hour	mg. 17-KS /hour	cc. urine /hour	mg. 17-KS /hour	cc. urine /hour	mg. 17-KS /hour
Control days	1	115	1.23	58	2.29	202	1.09
	2	120	1.11	71	2.00	142	0.89
	3	53	0.82	86	2.06	57	0.58
	1	187	1.40	38	2.09		
	2	183	1.21	46	1.43		
	3	96	1.22	32	1.12		
1000 cc. of water forced	1	395	1.37	110	2.07	73	0.76
	2	910	1.17	474	1.84	430	0.84
	3	132	1.07	160	1.59	453	0.94
	1	265	1.20	80	2.20		
	2	623	1.54	440	2.10		
	3	96	1.05	240	1.79		
Light exercise	1	395	1.45	406	2.86	370	0.74
	2	360	0.85	530	2.80	172	0.67
	3	237	1.43	133	2.10	385	
Heavy exercise	1	310	1.34	150	2.44		
	2	134	0.82	32	0.46		
	3	55	1.06	160	2.47		
	1	468	1.35	157	2.61		
	2	148	0.86	63	1.26		
	3	44	0.90	289	2.24		

in volumes of urine no greater than 20 to 40 cc. The data in Table 3 show that subject C excreted a smaller volume of urine during the post-exercise hour than during the exercise, although the excretion of total 17-ketosteroids following exercise was greater than that during exercise. Subject W showed a greater urinary output during the post-exercise hour than during exercise. However, on control days, volumes of urine equal to or smaller than those excreted during the heavy exercise were accompanied by 17-ketosteroid outputs approximately two to four times those observed during the exercise hour.

Comparisons of 17-ketosteroid output levels, determined on the same

sample by both routine total and chromatographic methods, are graphically illustrated in Figure 9. It was noted that on days when the large urinary volumes resulting from high fluid intake were assayed routinely the 17-ketosteroid levels were higher than when the samples were assayed chromatographically. There was a distinct difference in the patterns of total 17-ketosteroids routinely determined on control days and on diuresis days, the 17-ketosteroid determination during the second hour being

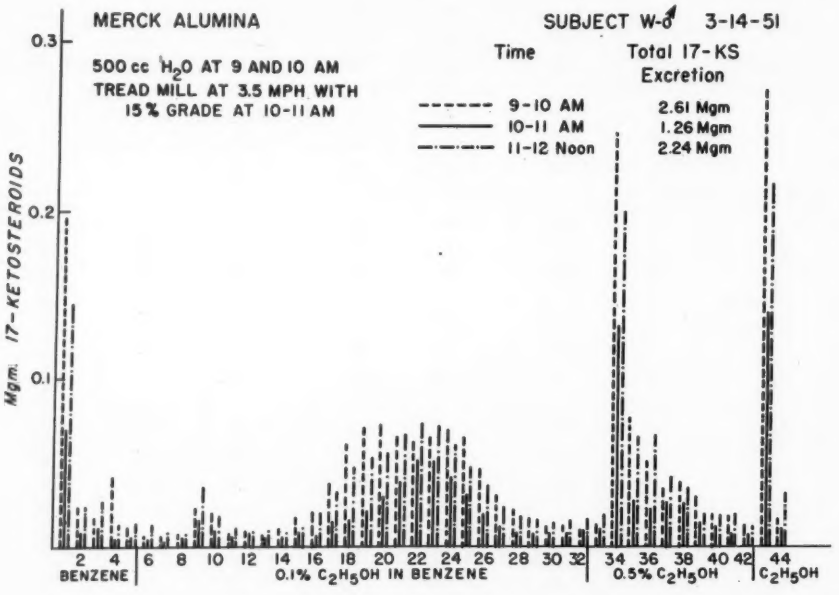

FIG. 8. 17-Ketosteroid patterns resulting from the chromatographic fractionation of Merck alumina of extracts of urine excreted during three consecutive hours. Fluid was forced at the beginning of the first and second hours, and heavy exercise was performed on the treadmill during the second hour. (Subject W.)

greater than during the first hour on diuresis days. This difference is not prominent when the total 17-ketosteroids are determined after chromatography, the patterns of excretion tending to resemble one another on the control and the diuresis days.

DISCUSSION

The blood eosinophils in the 2 subjects studied fluctuated considerably, but on the average they followed a pattern with the values falling in the morning and rising in the afternoon. The two- to four-hour delay in the response of eosinophils to the 11-oxygenated adrenal cortical hormones

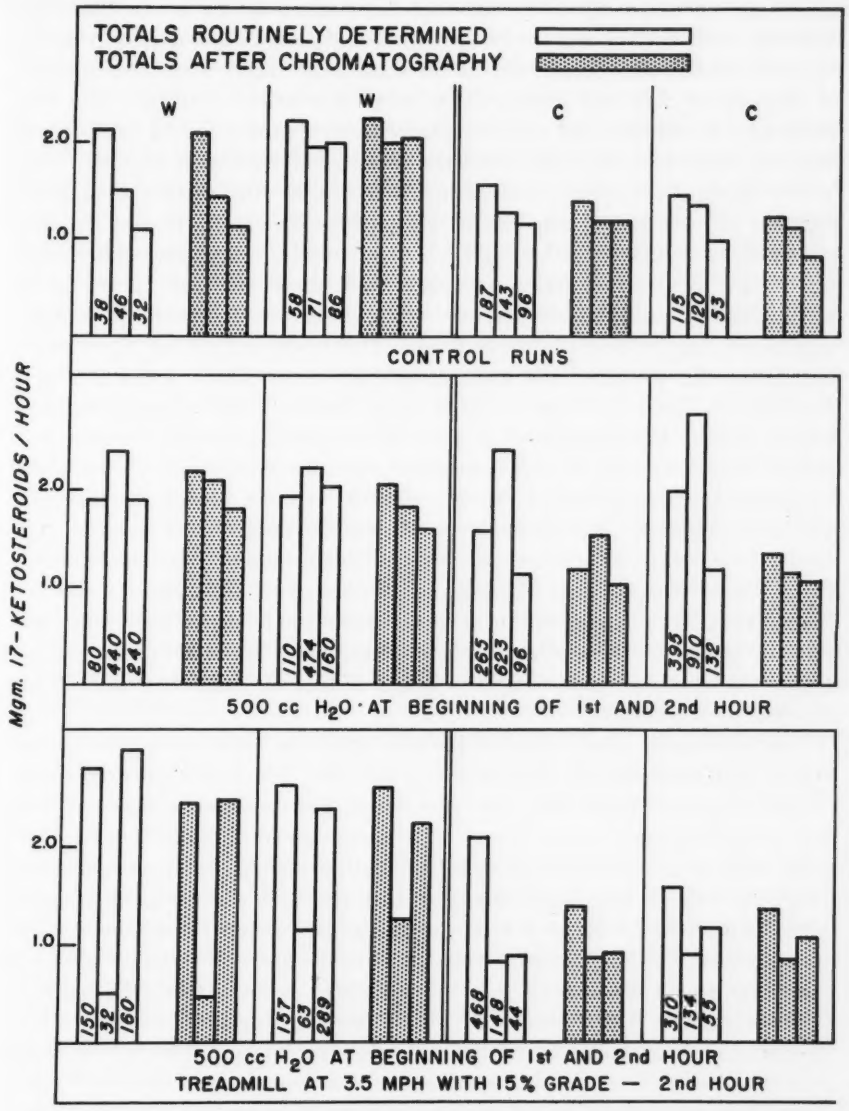

FIG. 9. Comparison of 17-ketosteroid totals determined before and after chromatographic fractionation of sets of three consecutive hourly urinary extract specimens under three different conditions. The figures at the base of each column indicate the number of cubic centimeters of urine in which the determined amount of 17-ketosteroids was contained.

(25) may be responsible for the mid-day values being lower than the morning values. The relatively low eosinophil levels noted just prior to exercise, which was performed at 10 A.M., may imply that anticipation of this event was accompanied by adrenal cortical activity. The low eosinophil levels after the exercise imply further activation of the adrenal cortices. This is in harmony with the findings of Renold *et al.* (26).

The qualitative chromatographic fractionation studies of the 17-ketosteroids did not reveal significant fluctuations in any particular fraction in any of the samples. Rather, all fractions tended to vary upward or downward together. An inconstant exception to this was the peak, presumably containing dehydroisoandrosterone, which at times decreased to a more significant degree than the other peaks. This could not be correlated with any particular phase of the experiments, however. These findings are in contrast to those of other workers using similar chromatographic techniques. Thus, Dingemanse *et al.* (17) demonstrated marked increases in certain fractions and the appearance of new 17-ketosteroids in the urine of patients with malignant adrenal cortical tumors. Other workers (18, 19) have reported deviations from previously determined normal patterns, with relative increases of certain 17-ketosteroids and decreases of other, in adrenal cortical virilism, acute illnesses and malignant disease. The data of the present report, however, are considered basically not comparable to those of the other workers because of the nature of the experiments, the short periods of urine collection, and the fact that all subjects studied were healthy.

The eosinophil data collected indicate that the activity of the adrenal cortex increased during the periods of exercise. The 17-ketosteroid excretion during this period, however, decreased. This is highly suggestive that many of the forerunners of these substances are completely broken down and utilized by the tissues in great quantities during the increased metabolic activity of muscular exercise. The rebound excretion of 17-ketosteroids following exercise harmonizes with this concept. That the qualitative pattern of 17-ketosteroid excretion during rest and during exercise is the same, suggests that the utilization of these substances during exercise is an exaggeration of a condition which exists during rest. The possibility of a greatly exaggerated hypersecretion of the adrenal cortex overriding this mechanism and causing qualitative changes in the urinary 17-ketosteroids is recognized, and has been previously suggested by Mason and Kepler (5).

In interpreting the significance of the decrease in 17-ketosteroid excretion upon exercise, the possibility that this is merely due to a decrease in renal clearance of these substances resulting from alterations in blood flow cannot be overlooked. No actual data are available on the renal

clearance of 17-ketosteroids, but a number of observations lead to the assumption that clearance is not actually impaired with muscular exercise. Koch (27) and Mason (28) have found that the quantities of free or esterified 17-ketosteroids contained in peripheral blood at any one time are so small that they cannot be detected by either biologic or chemical means. Samuels, McCaulay and Sellers (14) were unable to detect conjugation of these substances with the blood proteins. It is quite apparent, however, that over a period of time, relatively large quantities of 17-ketosteroids are carried in the blood and excreted by the kidneys. Huis in't Veld and Querido (29) demonstrated that the kidney is not necessary for the esterification of the 17-ketosteroids, and that normal excretion levels of these substances are maintained during extreme renal insufficiency. These observations have led to the concept that in regard to renal excretion, the 17-ketosteroids are no-threshold substances which are cleared principally if not exclusively by filtration. The suggestion that there is no threshold for the 17-ketosteroids was also advanced by Dorfman and Hamilton (30), who noted that injection of androgenic steroids into eunuchs, who presumably had a need for these substances, was followed by the prompt appearance of their conversion products in the urine.

Radigan and Robinson (31) have shown that the glomerular filtration rate, as indicated by the inulin clearance, is not significantly altered in subjects walking on a treadmill at 3 m.p.h. with a 5 per cent grade for eighty minutes. Since the clearance of a filtered substance is apparently not affected by the alterations of kidney circulation accompanying a degree of muscular exercise comparable to that performed in the present experiments, it is concluded that the decrease in 17-ketosteroid excretion which was noted indicated increased metabolic utilization of these substances or their precursors.

The data presented are not adequate to allow postulating the mechanism by which the 17-ketosteroid precursors are utilized in muscular metabolism. Further investigation of the form in which they are secreted by the adrenal cortex and the actual metabolism of these substances is clearly indicated.

The apparent rapid clearance of the 17-ketosteroids is in distinct contrast to the slow clearance of the C-21 adrenal cortical steroids noted by Corcoran and Page (32).

The present report adds to the controversy over the possible correlation between the total urinary 17-ketosteroids, and the total urine volume. Pincus (33) was concerned over the possibility of excess fluid "washing out" increased amounts of 17-ketosteroids, and by alternately exposing an individual to diuresis and to psychologic stress, he showed a complete absence of correlation between the 17-ketosteroid excretion level and the

volume of urine excreted over periods of from four to six hours. Other workers (34, 35) failed to note correlations between 17-ketosteroid content and total volume of 24-hour urine specimens. However, McHenry *et al.* (36) reported correlations between 24-hour urine volume and 17-ketosteroid output in 1 individual. Danford and Danford (37) demonstrated positive correlations between urine volume and 17-ketosteroid output in rabbits. In these latter two instances, no attempt was made to purify the 17-ketosteroids before their colorimetric determination. Pincus (33), however, removed nonketonic material from each of his specimens with the Girard reaction. Our data indicate that over short periods of time, relatively large volumes of urine tend to contain relatively large quantities of 17-ketosteroids as determined routinely without purification; but when these specimens are submitted to the purifying process of chromatography, the 17-ketosteroid totals in the presence of diuresis do not differ significantly from those in the absence of diuresis. Thus, large volumes of urine excreted over short periods of time apparently contain an increased amount of nonspecific chromogenic material which combines with the Zimmermann reagents to give falsely high colorimetric readings. The process of chromatography at least partially inactivates these chromogens, allowing a more accurate determination of the total 17-ketosteroids.

The failure of diuresis to produce an increase in 17-ketosteroid excretion lends further support to the theory that these substances have no renal threshold. The apparent correlation between urine volume and 17-ketosteroids determined routinely on urine excreted over short periods of time, and the apparent lack of correlation between 24-hour urine volume and 17-ketosteroid content (when determined routinely) may be explained by the hypothesis that the nonspecific chromogens are threshold substances, the excretion of which increases temporarily with diuresis. The importance of applying some purifying procedure in determining 17-ketosteroids is clearly indicated.

SUMMARY AND CONCLUSIONS

The character of the participation of the individual 17-ketosteroid components in the short-term variations in total urinary 17-ketosteroids has been studied by a chromatographic fractionation technique. The method devised, using alumina as the adsorbent, is suitable for evaluating the hourly amounts of 17-ketosteroid material excreted.

Under control conditions, studies on 1 female and 2 male subjects demonstrated slight variations in the kind and amount of 17-ketosteroids excreted by different subjects and by the same subject at different times. Forcing 1,000 cc. of water did not significantly alter the qualitative excretion pattern. Significant alterations in the excretion of those 17-ketoster-

oids closely related to adrenal cortical products were not noted. Rather, all 17-ketosteroids showed increases and decreases of relatively similar proportions.

Walking for one hour on a motor-driven treadmill with a 15 per cent grade at 3.5 m.p.h. significantly decreased the total 17-ketosteroid output, all component steroids being reduced proportionately. These data have been tentatively interpreted to mean that heavy exercise increases utilization of 17-ketosteroid precursors.

Further data derived from the chromatographic technique indicate that diuresis, although not causing an actual increase in 17-ketosteroid excretion, may over short periods of time cause increased excretion of nonspecific chromogens which react with the Zimmermann reagents to give falsely high 17-ketosteroid values.

Acknowledgment

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INHIBITION OF DESOXYCORTICOSTERONE-INDUCED PATHOLOGIC CHANGES BY ADRENOCORTICOTROPIC* HORMONE AND CORTISONE**†

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DESPITE recent advances in the treatment of the collagen diseases in man, their pathogenesis remains obscure. Pathologic lesions strikingly similar to those found in humans have been produced in animals by a wide variety of technics (1–21). Selye has claimed the production of typical lesions in animals following the administration of desoxycorticosterone acetate (DCA) (1–7), lyophilized anterior pituitary (LAP) (6, 8), compound S of Reichstein (10) and growth hormone (11, 12). He has concluded (7) that the collagen disorders in man result from a maladaptation to stress in which adrenocortical hyperactivity occurs, in response to excessive stimulation by the anterior pituitary.

On the other hand, studies in this laboratory (22, 23) indicate that the administration of DCA to the rat induces a relative state of adrenocortical insufficiency by inhibiting the output of pituitary adrenocorticotropic hormone (ACTH) and by antagonizing, at a target-cell level, the action of the ACTH-induced secretion of the adrenal cortex.

Desoxycorticosterone induces insulin hypersensitivity (22), decreased brain excitability and hypernatremia (24) in the rat. These toxic actions are inhibited or prevented if ACTH, adrenocortical extract (ACE) or cortisone is administered simultaneously with DCA (22, 25). The demonstration by Hench *et al.* (26, 27) that ACTH and cortisone are of therapeutic benefit in the collagen diseases in man stimulated us to test the possibility that ACTH and cortisone would also inhibit desoxycorticosterone-induced periarteritis nodosa, interstitial myocarditis and glomerular nephritis. Experimental proof of such an inhibition has been presented in preliminary reports from this laboratory (28–30). These observations, to-

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gether with additional confirmatory series of experiments, are now presented in detail.

METHODS

The animals employed were male rats from the Sprague-Dawley farm. Twelve 15-mg. pellets of DCA[1] were uniformly distributed throughout the subcutaneous tissue of the back of the rat. It has been demonstrated that approximately 0.2 mg. of DCA is absorbed from each pellet per day. The dose of ACTH[2] is expressed in all instances in U.S.P. units (one U.S.P. unit is the activity equivalent to 1.0 mg. of the International Standard, La-1-A). Preparation 64A, employed in Experiments 2A and 2B, has 1.8 U.S.P. units per mg.; it has a posterior pituitary pressor activity of 0.06 U.S.P. unit per U.S.P. unit of ACTH activity. Preparation 74AX, employed in Experiment 6A, has 0.5 U.S.P. unit of ACTH activity per mg.; it has a posterior pituitary pressor activity of less than 0.06 U.S.P. unit per U.S.P. unit of ACTH activity. Preparation 83ASI, employed in Experiment 6B, has 1.6 U.S.P. units of ACTH activity per mg.; it has a posterior pituitary pressor activity of less than 0.01 U.S.P. unit per U.S.P. unit of ACTH activity. ACTH was dissolved in a 0.9 per cent aqueous solution of sodium chloride made to 0.01 N with hydrochloric acid; each dose was injected subcutaneously in a volume of 0.1 or 0.2 ml. Each ml. of ACE,[3] used in Experiment 4, was equivalent in biologic activity (muscle-work test) to 2 mg. of cortisone. Cortisone acetate,[4] employed in Experiments 6A and 6B, was suspended in 10 per cent ethanol; each dose was injected subcutaneously in a volume of 0.2 ml. Posterior pituitary substance (Pitressin, Parke-Davis and Company, 20 U.S.P. pressor units per ml.) was diluted with 0.9 per cent sodium chloride solution to a concentration of 0.1 unit per 0.2 ml.; this volume was injected subcutaneously.

The tissues were fixed in a mixture of 95 parts of Zenker's solution and 5 parts of glacial acetic acid. The sections were stained according to Goldner's modification of the Masson trichrome technic (31).

Microscopic sections of heart, kidney and pancreas were examined. The sections were graded from zero to four plus on the basis of extent and severity of the pathologic changes. A value of zero indicates that no pathologic change was observed; that of four plus, extensive and severe lesions. In the pancreas, the changes noted were confined to the blood vessels. Alterations were observed in both the blood vessels and the interstitium of the heart. Pathologic changes were seen in the glomerular, tubular, inter-

[1] Kindly supplied by Dr. Fred Houghton of Ciba Pharmaceutical Products, Inc.

[2] Gift of Dr. E. E. Hays of Armour Laboratories.

[3] Lipo-Adrenal Cortex, gift of Dr. H. H. Hailman of The Upjohn Company.

[4] Cortone, kindly supplied by Dr. Augustus Gibson of Merck & Co., Inc.

stitial and vascular components of the kidney. In the heart and in the kidney, the grading was an over-all evaluation of the pathologic changes in all components of these organs.

RESULTS

Experiment 1. Twenty-one rats, each weighing from 130 to 160 grams, were divided into four groups. The six animals in Group 1 were given water and the six animals in Group 2, 0.9 per cent sodium chloride solution, to drink; the four animals in Group 3 were unilaterally nephrectomized[5] and given water to drink; the five animals in Group 4 were unilaterally nephrectomized and given 0.9 per cent sodium chloride solution to drink. Four days after unilateral nephrectomy, DCA pellets were implanted subcutaneously in three rats of Group 1, three of Group 2, two of Group 3 and three of Group 4. Some animals in each group were sacrificed on the fourteenth day after DCA implantation. The remainder were sacrificed on the twenty-eighth day

No pathologic changes were observed except a minimal amount of interstitial myocarditis in two DCA-treated animals (one animal of Group 1 and one animal of Group 3).

Experiments 2A and 2B. In Experiment 2A, twelve rats, each weighing from 265 to 320 grams, were unilaterally nephrectomized and given 0.9 per cent sodium chloride solution to drink. Five days later DCA pellets were implanted in eight of the animals. Four of the DCA-treated rats were injected with ACTH, 1.0 U.S.P. unit thrice daily. One of the rats of the DCA-ACTH-treated group died as a result of accidental trauma on the twentieth day of hormone treatment. All animals were sacrificed 30 days after DCA implantation.

In Experiment 2B, started one week later, twelve rats were treated as in Experiment 2A.

The results are presented in Table 1. No pathologic changes were observed in the heart, kidney or pancreas of the untreated animals in Experiments 2A and 2B. DCA induced marked changes in the animals of Experiment 2A. Interstitial myocarditis was present in all animals treated with DCA alone. Focal glomerulitis of varying intensity was found in all except one animal. The pancreas of all rats treated with DCA alone exhibited a severe degree of periarteritis nodosa. The pathologic changes induced by DCA in Experiment 2B were less constant than those in Experiment 2A. The heart was the only organ to be consistently involved in both groups.

In Experiment 2A, concomitant administration of ACTH resulted in a

[5] The right kidney was removed in all instances.

TABLE 1. EXPERIMENTS 2A AND 2B. PATHOLOGIC CHANGES

Exp.	Treatment	Heart	Kidney	Pancreas
2A	Untreated	0	0	0
		0	0	0
		0	0	0
		0	0	0
	DCA (twelve 15-mg. pellets)	3+	3+	4+
		4+	2+	4+
		4+	2+	4+
		2+	0	3+
	DCA (twelve 15-mg. pellets) plus ACTH (1.0 U.S.P. unit 3 times daily)	0	0	1+
		1+	0	1+
		1+	2+	3+
2B	Untreated	0	0	0
		0	0	0
		0	0	0
		0	0	0
	DCA (twelve 15-mg. pellets)	1+	0	2+
		1+	0	0
		2+	0	0
		1+	2+	4+
	DCA (twelve 15-mg. pellets) plus ACTH (1.0 U.S.P. unit 3 times daily)	1+	0	2+
		1+	0	2+
		1+	0	0
		0	0	0

striking reduction in the severity of the pathologic changes induced by DCA. In Experiment 2B, treatment with ACTH appeared to have no significant effect.

Experiment 3. Twelve rats, each weighing from 290 to 350 grams, were unilaterally nephrectomized and given 0.9 per cent sodium chloride solution to drink. Five days later DCA was implanted into each of the twelve rats. The following day the rats were injected intravenously with 1.0 ml. of sterile horse serum per 100 grams of body weight. In addition, six untreated intact rats, each weighing from 320 to 370 grams, were injected with the same dose of horse serum. Ten days later both groups were again injected intravenously with the same dose of horse serum. One of the DCA-treated rats had a reaction characterized by severe respiratory difficulty

similar to that which occurs in acute anaphylactic shock in the guinea pig. One DCA-treated and one control rat exhibited a mild reaction manifested by wheezing. Ten days later the same dose of horse serum was again injected intravenously. Various degrees of asthmatic-like reactions immediately followed the injection. The reactions were graded as minimal to severe. The results are presented in Table 2. DCA increased the incidence and the severity of the reactions to horse serum. Epinephrine was injected intraperitoneally into some of the animals exhibiting moderate to severe reactions; striking amelioration followed its administration in all instances.

TABLE 2. EXPERIMENT 3. EFFECT OF DCA ON REACTION TO THIRD DOSE OF HORSE SERUM

Treatment	No. of rats	Severity of reaction
DCA	3	Severe
	3	Moderate
	2	Mild
	4	Minimal
	0	No reaction
Controls	0	Severe
	0	Moderate
	2	Mild
	1	Minimal
	2	No reaction

Four control and four DCA-treated animals were sacrificed. No pathologic changes were observed in the heart, kidney or pancreas of the animals treated with horse serum alone. In the group given DCA plus horse serum, two animals had a 1 plus involvement of the pancreas and one animal had a 2 plus change in the heart and in the kidney.

Experiment 4. Fifteen rats, each weighing from 249 to 330 grams, were unilaterally nephrectomized, implanted with DCA and given 0.9 per cent sodium chloride solution to drink. The animals were exposed to cold (2° to 8°C.) in individual cages eight hours daily. Eight of the rats were given no additional treatment. The remainder were injected with 0.5 ml. of ACE twice daily. The animals were sacrificed 30 days after DCA implantation.

In the group treated with DCA alone, one animal had a 1 plus involvement of the kidney and of the pancreas; two animals, 1 plus involvement of the pancreas; and one animal, 1 plus involvement of the heart. In the group given ACE, two animals had 1 plus involvement of the pancreas.

Experiment 5. Fifty-one rats, each weighing from 100 to 150 grams were given 1.5 per cent sodium chloride solution to drink. Twenty-one of the

animals were implanted with DCA; the remainder were kept as controls. Four treated and four control rats were sacrificed at 15, 36, 58, and 86 days after implantation. The remainder of the animals were sacrificed at 144 days after implantation. At 15 days, no pathologic changes were observed. At 36 days, one of the DCA-treated animals had a 3 plus degree of interstitial myocarditis. No pathologic changes were seen in the controls. At 58 days, three of the DCA-treated animals had lesions. One animan had 1 plus periarteritis nodosa in the pancreas; one animal, 1 plus interstitial myocarditis; and one animal, 1 plus involvement of the kidney and of the heart and 2 plus involvement of the pancreas. No pathologic changes were seen in the controls. At 86 days, two of the DCA-treated animals had lesions. One animal had a 2 plus periarteritis nodosa in the pancreas and 1 plus interstitial myocarditis, and one animal had a 1 plus involvement of the kidney. No lesions were seen in the controls. At 144 days, four of five DCA-treated and two of fifteen control rats exhibited pathologic changes. An analysis of the changes observed at 144 days is presented in Table 3.

TABLE 3. EXPERIMENT 5. PATHOLOGIC CHANGES IN RATS ON 1.5% NaCl FOR 144 DAYS

	Animal No.	Heart	Kidney	Pancreas
	1–12	0	0	0
Controls	13	0	2+	3+
	14	0	1+	0
	15	0	0	0
	1	0	2+	3+
	2	0	2+	2+
DCA	3	0	0	0
	4	1+	0	0
	5	0	2+	4+

Experiments 6A and 6B. Information regarding the nature of the drinking fluid, hormone administration, body weight changes and results of treatment of each group of rats is presented in Table 4. Unilateral nephrectomy, DCA implantation and substitution of 0.9 per cent sodium chloride solution for drinking water were carried out on the same day. In addition to the treatment presented in Table 4, all animals in Experiments 6A and 6B were electroshocked every two to three days. The measurements of electroshock seizure threshold were conducted in connection with a separate study on brain excitability (23, 32). The animals in Experiment 6A

TABLE 4. EXPERIMENTS 6A AND 6B. TREATMENT AND PATHOLOGIC CHANGES

Expt.	Group	No. of rats	Unilateral nephrectomy	Drinking fluid	DCA, 15-mg. pellets	ACTH, total daily dose, U.S.P. units	Cortisone, total daily dose, mg.	Initial body wt. (avg. ± S.E.) Gm.	Final body wt. (avg. ± S.E.) Gm.	Extent and severity of pathologic changes			Degree of pathologic change	
										Heart	Kidney	Pancreas	Avg.	S.E.
6A	1	4	No	H_2O	None	None	None	350 ± 19	380 ± 25	0 0 1+ 0	0 0 0 0	0 0 0 0		
	2	5	No	NaCl	None	None	None	350 ± 17	375 ± 16	0 0 0 0 0	0 0 0 1+ 0	0 0 0 1+ 1+	0.18+	±0.07
	3	6	Yes	NaCl	None	None	None	342 ± 18	368 ± 17	0 0 0 0 0 0	0 0 0 0 0 0	2+ 0 0 0 2+ 0		
	4	5	Yes	NaCl	12	None	None	337 ± 9	305 ± 18	2+ 2+ 3+ 2+ 4+	4+ 4+ 4+ 2+ 2+	4+ 4+ 4+ 2+ 3+	3.06+	±0.25
	5	6	Yes	NaCl	12	3(3)*	None	345 ± 6	322 ± 9	0 1+ 1+ 2+ 4+ 2+	0 2+ 0 0 2+ 2+	3+ 1+ 1+ 3+ 2+ 3+	1.61+	±0.28
	6	6	Yes	NaCl	12	0.5(1)	None	306 ± 5	278 ± 14	0 0 0 1+ 0 0	2+ 0 0 1+ 1+ 3+	2+ 1+ 2+ 1+ 2+ 4+	1.11+	±0.28
	7	5	Yes	NaCl	12	0.05(1)	None	344 ± 15	348 ± 12	1+ 3+ 4+ 1+ 4+	3+ 4+ 2+ 0 2+	2+ 4+ 4+ 1+ 4+	2.60+	±0.36
	8	4	Yes	NaCl	None	3(3)	None	325 ± 20	313 ± 20	0 0 0 0	0 0 2+ 0	0 1+ 0 1+	0.33+	±0.19
	9	5	Yes	NaCl	12	None	2(2)*	336 ± 6	260 ± 8	0 1+ 1+ 1+ 0	0 2+ 1+ 1+ 2+	2+ 3+ 0 1+ 0	1.27+	±0.30
	10	6	Yes	NaCl	None	None	2(2)	333 ± 11	270 ± 12	0 0 0 0 0 0	0 0 0 2+ 0 0	0 0 0 0 1+ 0	0.17+	±0.12

TABLE 4—*continued*

Expt.	Group	No. of rats	Unilateral nephrectomy	Drinking fluid	DCA, 15-mg. pellets	ACTH, total daily dose U.S.P. units	Cortisone, total daily dose, mg.	Initial body wt. (avg. ± S.E.) Gm.	Final body wt. (avg. ± S.E.) Gm.	Extent and severity of pathologic changes				
										Heart	Kidney	Pancreas	Degree of pathologic change	
													Avg.	S.E.
6B	11	7	Yes	NaCl	None	None	None	263 ± 5	353 ± 6	0 0 0 0 3+ 0 0	0 0 0 1+ 0 2+ 0	0 0 0 0 2+ 3+ 1+	0.57+	±0.22
	12	7	Yes	NaCl	12	None	None	273 ± 4	319 ± 8	2+ 1+ 1+ 1+ 2+ 2+ 2+	3+ 0 3+ 3+ 2+ 2+ 3+	3+ 0 4+ 3+ 4+ 4+ 0	2.14+	±0.28
	13	7	Yes	NaCl	12	0.001(1)	None	266 ± 6	298 ± 11	1+ 2+ 1+ 2+ 2+ 0 0	3+ 2+ 2+ 2+ 0 2+ 2+	4+ 3+ 3+ 3+ 0 3+ 2+	1.86+	±0.25
	14	7	Yes	NaCl	12	0.01(1)	None	271 ± 6	312 ± 8	0 3+ 1+ 3+ 2+ 1+ 0	† 1+ 3+ 4+ 4+ 0 2+	2+ 3+ 4+ 4+ 2+ 0 2+	2.05+	±0.32
	15	6	Yes	NaCl	12	10(5)	None	277 ± 9	256 ± 11	0 † 2+ 0 0 0	2+ † 2+ 1+ 2+ 0	1+ † 2+ 3+ 2+ 2+	1.27+	±0.27
	16‡	7	Yes	NaCl	12	0.5(1)	None	274 ± 7	323 ± 9	0 0 1+ 0 0 † 0	0 0 2+ 1+ 2+ † 1+	0 0 4+ 1+ 1+ † 3+	0.89+	±0.28
	17	7	Yes	NaCl	12	None	0.1(1)	263 ± 12	307 ± 13	3+ 2+ 2+ 3+ 3+ 3+ 0	3+ 3+ 2+ 3+ 4+ 3+ 2+	3+ 3+ 2+ 3+ 3+ 2+ 3+	2.62+	±0.18

* Numbers in parentheses indicate number of doses per day.
† Tissue sample lost.
‡ Posterior pituitary, 0.1 unit 5 times daily.

were sacrificed 38 to 40 days after DCA implantation. The animals in Experiment 6B were sacrificed 41 to 43 days after DCA implantation.

The average degree of pathologic change with the standard error for each group is presented in Table 4. An example of the calculation of the average follows: in the controls (Groups 1, 2 and 3 combined) of Experiment 6A the grading of the individual organs resulted in thirty-nine zero ratings, four 1 plus, two 2 plus, and no 3 or 4 plus ratings. The total, $8[(39\times0)$

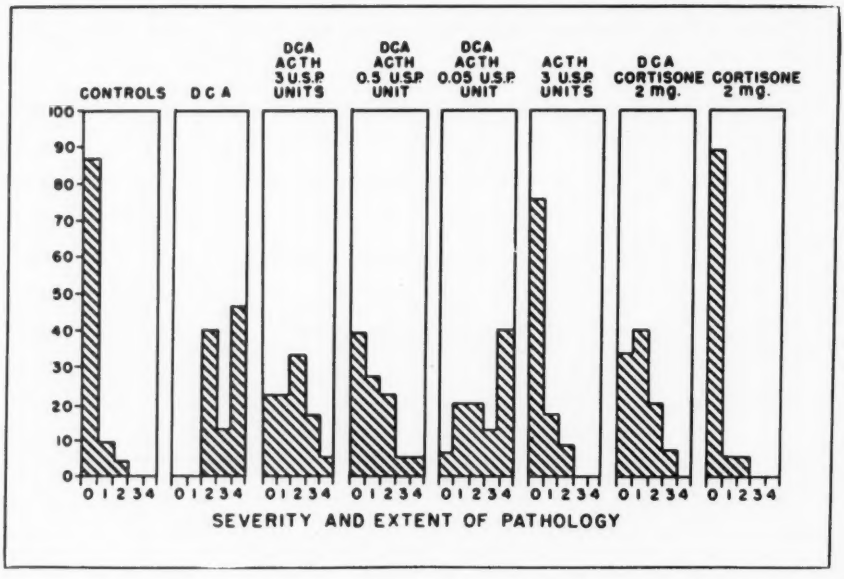

FIG. 1. Frequency distribution of severity and extent of pathologic changes in Experiment 6A. (See text for explanation.)

$+(4\times1)+(2\times2)+(0\times3)+(0\times4)]$, divided by the number of organs examined, 45, equals the average, 0.18.

It can be seen from Table 4 that a low incidence of pathologic lesions occurred in the three groups of controls [intact water controls (Group 1), intact sodium chloride controls (Group 2) and unilaterally nephrectomized sodium chloride controls (Group 3)] in Experiment 6A. In contrast, in the DCA-treated rats (Group 4), pathologic lesions were numerous in the heart, kidney and pancreas. Interstitial myocarditis, glomerular and interstitial nephritis and periarteritis nodosa of the pancreatic vessels of varying severity were observed. In the animals treated with ACTH alone (Group 8) and with cortisone alone (Group 10) the incidence of lesions was not significantly different from that in the controls. ACTH in a dose of

1.0 U.S.P. unit thrice daily (Group 5) or 0.5 U.S.P. unit once daily (Group 6), given continuously from the time of DCA implantation, decreased the incidence and severity of the pathologic changes. However, the animals given the combined treatments had a significantly higher incidence of lesions than the controls. ACTH in a dose of 0.05 U.S.P. unit once daily (Group 7) failed to inhibit the toxic action of DCA on tissues. Cortisone in a dose of 1 mg. twice daily (Group 9) significantly reduced the severity of the pathologic changes.

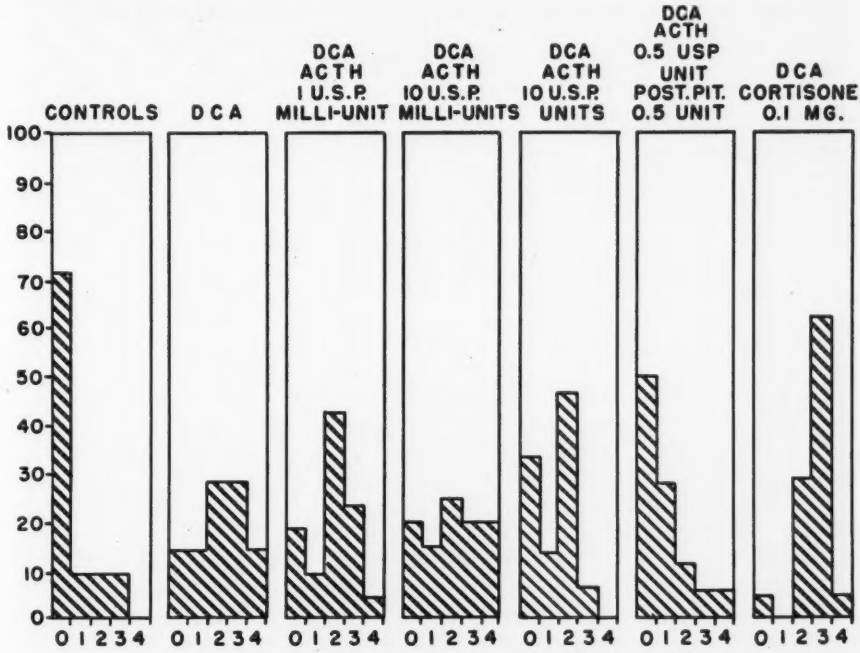

FIG. 2. Frequency distribution of severity and extent of pathologic changes in Experiment 6B. (See text for explanation.)

In Figure 1 a composite frequency distribution of the lesions among the various groups is presented. The number of ratings were totalled. For example, in the controls, three organs of each of fifteen rats (combination of three groups of controls) were evaluated, making a total of 45 ratings. Thirty-nine zero ratings, four 1 plus ratings, two 2 plus ratings, and no 3 or 4 plus ratings give a frequency distribution of 87, 9, 4, 0 and 0 per cent, respectively. This was calculated for each group and graphed in Figure 1. A shift of the frequency distribution to the right indicates an increase in severity of lesions; a shift to the left, a decrease. The marked effect of 3

U.S.P. units of ACTH, of 0.5 U.S.P. unit of ACTH and of 2 mg. of cortisone in reducing the severity of the pathologic lesions induced by DCA is illustrated in this figure. Comparative statistical analyses by the χ^2 test are presented in Table 5.

The results of Experiment 6B are also presented in Table 4. A slightly higher average incidence of pathologic changes occurred in the controls in this experiment (Group 11) as compared to the controls in Experiment 6A

TABLE 5. P VALUES OBTAINED FROM χ^2 ANALYSIS OF EXPERIMENTS 6A AND 6B

Expt.	Treatment / Treatment	Controls	DCA
6A	DCA	<0.001	—
	DCA+ACTH, 3 U.S.P. units	<0.001	0.02
	DCA+ACTH, 0.5 U.S.P. unit	<0.001	0.01
	DCA+ACTH, 0.05 U.S.P. unit	<0.001	0.6
	ACTH, 3 U.S.P. units	0.6	—
	DCA+Cortisone, 2 mg.	<0.001	0.01
	Cortisone, 2 mg.	0.98	—
6B	DCA	<0.001	—
	DCA+ACTH, 0.5 U.S.P. unit+Post. Pit.	0.4	0.02
	DCA+ACTH, 10 U.S.P. units	0.01	0.05
	DCA+ACTH, 0.01 U.S.P. unit	—	0.90
	DCA+Cortisone, 0.1 mg.	—	0.3

(Groups 1, 2 and 3). The difference is not significant. DCA alone (Group 12) induced marked pathologic changes, which included interstitial myocarditis and periarteritis nodosa in the heart, glomerular and interstitial nephritis and periarteritis nodosa in the kidney, and periarteritis nodosa in the pancreas. ACTH in a dose of 1 U.S.P. milliunit (one thousandth of a unit) (Group 13) or 10 U.S.P. milliunits (Group 14) once daily, given continuously from the time of DCA implantation, had no influence upon the

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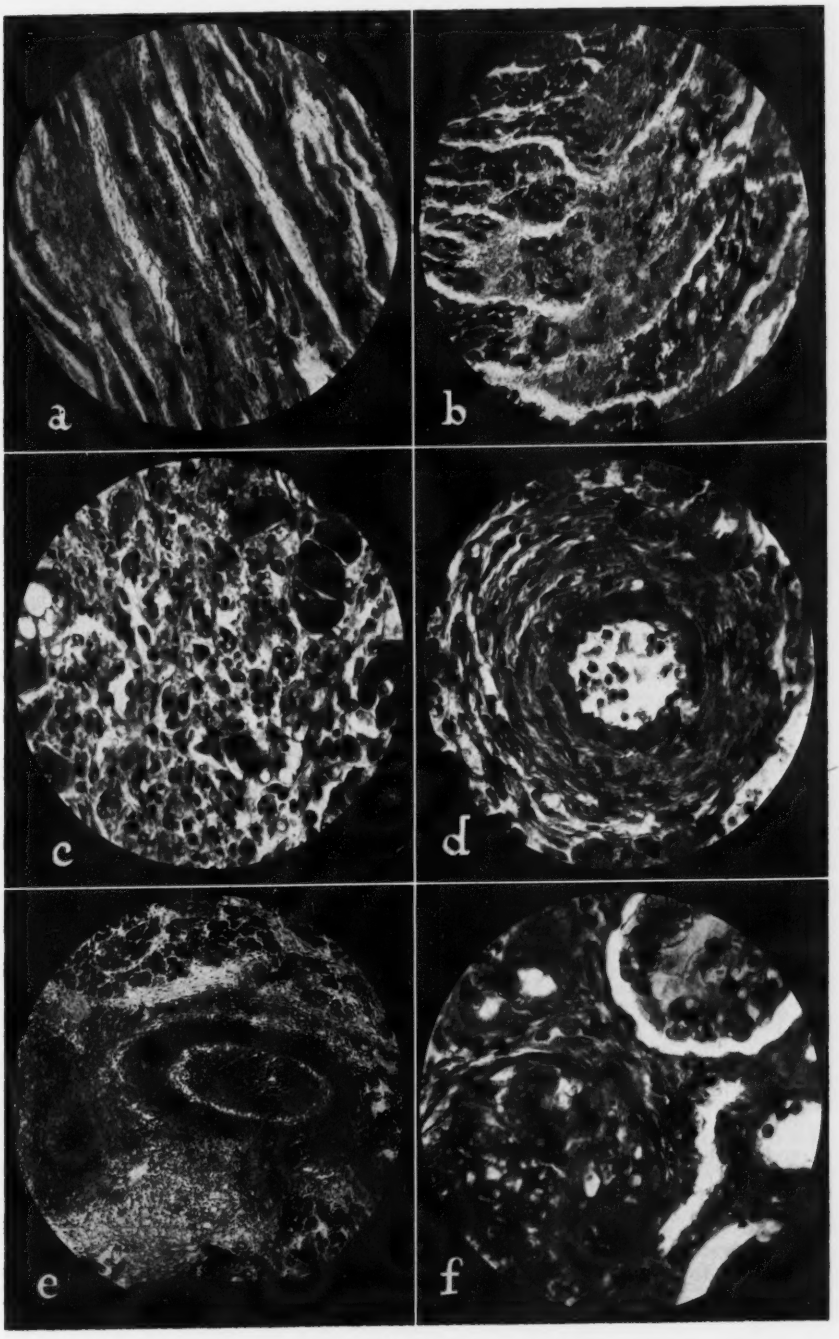
FIG. 3. Examples of pathologic changes induced by DCA in rats. *3a*) Acute interstitial myocarditis. Note the infiltration of acute inflammatory cells, and degenerative changes in muscle fibers. Low power. *3b*) Granuloma in the myocardium. Low power. *3c*) High power view of area in *3b*) Note the presence of mononuclear cells, macrophages and giant cells. *3d*) High power view of a small artery in the pancreas, with changes evident in all layers of its wall, as well as intense perivascular reaction. *3e*) Severe periarteritis nodosa in the pancreas. Low power. *3f*) High power view of 2 adjacent glomeruli. Note process of acute glomerulitis, with adjacent normal glomerular structure for comparison.


a
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severity of the lesions induced by DCA alone. ACTH in a dose of 0.5 U.S.P. unit once daily (Group 16)[6] significantly reduced the severity and the incidence of lesions in DCA-treated rats. The number of lesions in the group given DCA plus 0.5 U.S.P. unit of ACTH was not significantly higher than that in the control group. ACTH, in a dose of 2 U.S.P. units five times daily (Group 15), also reduced the severity and the incidence of pathologic changes. However, in these animals the incidence of pathologic changes was significantly higher than in the untreated controls. In the animals given DCA plus cortisone in a dose of 0.1 mg once daily (Group 17), the over-all average degree of pathologic change was actually greater than in the rats given DCA alone (Group 12), but the difference is not statistically significant. Figure 2 illustrates the frequency distribution of lesions in Experiment 6B. Comparative statistical analyses by the χ^2 test are presented in Table 5. Figures 3a to 3f are microphotographs representative of some of the pathologic changes noted in these experiments.

DISCUSSION

Quantitative evaluation of DCA-induced lesions in rats. Three individuals graded the severity of the pathologic changes, independently, and without knowledge of the treatment. There was excellent agreement among the evaluations. In the few instances of disagreement, a final grading was based on the outcome of a discussion among the observers. In our experience, estimation of the degree of pathologic change presents no serious difficulties.

Reproducibility of DCA-induced lesions in rats. Despite the care with which certain experiments were controlled in regard to age, sex, environmental temperature, sodium intake and DCA treatment, there was a marked difference in the incidence and the severity of the pathologic changes. Fortunately, in any given experiment there was a high degree of uniformity among the rats of each experimental group. It is obvious that one or more variables, as yet not recognized, influence the development of lesions in DCA-treated rats.

Selye (33) states that " . . . females are usually a little more sensitive and the best results are obtained in rats of about 60 to 80 grams." All the rats employed in the experiments presented in this paper were males and most of them weighed about 300 grams. This may account for the fact that we did not obtain the high degree of reproducibility reported by Selye and his coworkers. Experimental work is under way to test this point. If DCA induced these lesions with a high degree of reproducibility in rats, the phenomenon might be seriously considered as a screening test for the evaluation

[6] It appears that Pitressin, given in addition to ACTH, did not influence the ability of the latter to inhibit the action of DCA. (Compare Groups 6 and 16, Table 4.)

of substances of potential therapeutic value in the mesenchymal diseases of man.

Ingestion of excessive sodium chloride (34) and unilateral nephrectomy (3) have been reported to increase the incidence of lesions in DCA-treated rats. The studies are suggestive, but far from conclusive. Unfortunately, the experiments of this report do not contribute to an evaluation of the importance of these factors. Adequately controlled studies designed to make such an evaluation possible are now being conducted.

Selye (4) has claimed that rheumatoid arthritis is produced in unilaterally nephrectomized rats which are given sodium chloride solution to drink, and treated with DCA. He has also reported that various additional stresses tend to aggravate these lesions. Of the large number of rats which have been unilaterally nephrectomized, given sodium chloride solution to drink, and implanted with DCA in this laboratory, not a single animal has manifested articular pathology. Harrison (13) was unable to produce arthritic changes with DCA. Pemberton (19) found that untreated as well as DCA-treated rats may manifest arthritic changes. Preston (14) has noted that suppurative arthritis occurs in rats infected with pleuropneumonia organisms. Of interest in this connection is the work of Dienes (35) who has isolated pleuropneumonia-like organisms from human synovial fluid and urine in cases of Reiter's syndrome (urethritis, conjunctivitis and arthritis). Infection may have played a major role in the production of arthritic changes in Selye's rats; it is of interest that a number of his rats succumbed from pneumonia.

Role of the pituitary-adrenocortical system in the etiology of mesenchymal tissue disease. The experiments reported herein confirm the earlier work of Darrow and Miller (15) and of Selye and co-workers (1–7) that DCA can induce pathologic changes in rats. The similarity between the pathologic changes produced in experimental animals by DCA treatment and those seen in the mesenchymal diseases led Selye to the sweeping generalization that hyperactivity of the adrenal cortex is an etiologic factor in the development of these disorders in man. The concept of the General Adaptation Syndrome (GAS) has been the subject of a review (7) and a text (36). The interested reader is referred to these publications for details. Suffice it here to quote Selye (7) that ". . . all experimental observations are most readily compatible with the view that during the general adaptation syndrome, certain hormones of the anterior pituitary and adrenal cortex are produced in excessive amounts in order to increase resistance; this defensive endocrine response is valuable in as much as it facilitates adaptation to stress (*e.g.*, infections, intoxications, nervous commotions, cold, *etc.*), but the resulting endogenous hormone overdosage may become the cause of certain cardiovascular, renal and joint diseases. Thus developed the concept that

many of the most common maladies of man are 'diseases of adaptation,' that is to say, the by-products of abnormal adaptive reactions of stress."

Actually, there is very little evidence to support the GAS concept. It is well established that stress increases the secretory activity of the adrenal cortex. However, it has not been demonstrated either in man or in experimental animals that stress (in the non-specific sense of the word), uncomplicated by DCA treatment, induces the development of collagen disease. Selye (7) has stated, without presentation of data, that " . . . partial kidney insufficiency produced by unilateral nephrectomy sensitizes animals to the development of nephrosclerosis, nephritis, hypertension and periarteritis nodosa, following chronic exposure to damaging agents, such as cold." Yet this same author reported elsewhere (37) that he was unable to induce pathologic changes characteristic of the collagen diseases of man (periarteritis nodosa, interstitial myocarditis and arthritis) in animals exposed to cold, injected with formalin or forced to exercise. "Aschoff bodies in the heart muscles, periarteritis of the intestinal vessels or arthritic changes, such as are produced by chronic overdosage with DCA, were not observed in any of the animals of this series." In Experiment 4 of the present report no significant degree of pathologic change occurred in animals treated with DCA and exposed to cold. The experiment does not support the thesis that cold stress sensitizes the animal to the development of periarteritis nodosa.

A real test of the value of a concept is its application to the prediction of new experimental facts. Meiklejohn (38, 39) concluded that there was nothing in the GAS concept which would have led one to anticipate the therapeutic effect of ACTH and cortisone in the collagen diseases in man. However, Selye creates the impression that he did indeed make such a prediction in the GAS concept. He states (36, p. 6), referring to twelve enumerated facts, two of which are presented below, that " . . . theory led us to them at a time when other approaches failed to do so." Two of the facts follow:

"(11) ACTH or gluco-corticoids (e.g., cortisone) inhibit the production of hyalinosis by crude pituitary extracts or mineralo-corticoids (e.g., desoxycorticosterone) in animals.

"(12) ACTH or gluco-corticoids exert a beneficial effect in the 'spontaneous' hyalinoses (rheumatic-allergic collagen diseases) of man."[7]

We have failed to find any statement in Selye's writings (published prior to the dramatic discovery of the Mayo group) which would indicate that the GAS concept was a basis for the prediction of facts (11) and (12). As will be discussed in detail later, in the GAS concept (7) ACTH was

[7] In the interest of historical accuracy (12) should precede (11).

considered to be the agent responsible for the development of lesions induced in rats by the administration of lyophilized anterior pituitary!

In reference to early work (4) in which it was demonstrated that in adrenalectomized animals arthritis developed much more easily than in intact controls, Selye states (36, p. 392) that, "This led us to assume that some adrenal principle normally antagonizes the arthritis-producing effect of DCA." Careful reading of the report referred to has failed to uncover such an assumption. Hench *et al.* (26, 27) were prompted to try ACTH and cortisone on the basis of the fact that certain types of stress (pregnancy, jaundice, general anesthesia, surgical trauma) produce temporary remissions in rheumatoid arthritis. This is incompatible with the GAS concept.[8]

Rheumatoid arthritis has been found to occur in patients with Addison's disease (40) and in panhypopituitarism (41), facts very difficult to reconcile with Selye's thesis that hyperfunction of the adrenal cortex is an etiologic agent in mesenchymal disease.

A refinement of the GAS concept specifies the nature of the secretion of the adrenal cortex. Selye (42) states that ". . . nephrosclerosis, renal hypertension, periarteritis nodosa and perhaps even some of the so-called rheumatic conditions are diseases of adaptation, due to an excessive or otherwise abnormal (*preferential formation of salt-active corticoids*) adrenal cortical response to non-specific damage." (Italics are those of the authors.) Any statement regarding the exact nature of the secretion of the adrenal cortex of man in health or disease is highly speculative at the present stage of development of adrenocortical physiology. Furthermore, no metabolic aberrations compatible with the elaboration of an excess of "salt-active" steroids by the adrenal cortex have been demonstrated to exist in patients with collagen disease. Finally, the experiments of the present report fail to support the notion that the concentrations of sodium or potassium in the body fluids are related to the severity of the pathologic changes induced. Rats treated with DCA and given 0.9 per cent sodium chloride solution to drink (Group 4, Table 6) had hypernatremia, hypokaliemia, high concentration of tissue sodium and low concentration of tissue potassium. Rats treated with DCA plus cortisone and given 0.9 per cent sodium chloride solution to drink (Group 9, Table 6) had the same degree of hypernatremia, an equally high concentration of tissue sodium, a more marked hypokaliemia and an even lower concentration of tissue potas-

[8] It is to be noted that Selye has recently modified the GAS concept and he states on page 589 of his textbook "Stress" (36) that "—we feel that experimental data agree with the conception that, depending upon conditions, *the hormonal response to stress can either sensitize or desensitize* the organism to the production of rheumatic changes." (Italics are those of the authors.) The GAS concept is most elastic indeed!

sium, yet these animals had a markedly lessened degree of pathologic change in their tissues compared with the animals given DCA alone. Since the pathologic changes were less severe in the animals whose electrolyte metabolism was most severely disturbed, it may be concluded that no simple relationship, as postulated by the GAS concept, exists between the mesenchymal diseases and electrolyte metabolism.

Lyophilized anterior pituitary (LAP) produces lesions similar to those induced by DCA (6, 8, 43). According to Selye (7), ACTH is most prob-

TABLE 6. EXPERIMENT 6A. PLASMA AND MUSCLE ELECTROLYTES

Group	Treatment	Plasma			Muscle			
		Na	K	Cl	H_2O	Na	K	Cl
		mM/Kg. plasma			%	mM/Kg. fat-free wet tissue		
1	Water	147.8 ±3.0	5.85 ±0.62	108.6 ±2.9	78.84 ±0.09	23.90 ±0.80	110.9 ±0.47	13.84 ±0.54
2	NaCl	147.5 ±3.1	5.16 ±0.53	111.8 ±5.3	79.16 ±0.21	22.28 ±0.91	111.4 ±2.38	14.12 ±1.02
3	NaCl, Nephrex	150.3 ±3.4	4.62 ±0.36	114.0 ±2.5	78.70 ±0.17	23.68 ±1.36	109.3 ±1.15	14.14 ±0.46
4	NaCl, Nephrex, DCA	153.2 ±3.9	4.70 ±0.57	109.2 ±1.7	78.92 ±0.52	40.15 ±0.41	88.25 ±0.82	17.71 ±1.15
9	NaCl, Nephrex, DCA, Cortisone, 2 mg.	153.0 ±4.1	3.43 ±0.24	120.4 ±9.3	79.64 ±0.36	43.78 ±1.47	85.42 ±1.64	20.16 ±0.37

ably the factor in LAP which stimulates the adrenal cortex to produce desoxycorticosterone-like substances, which in turn are responsible for the pathologic changes. However, Ingle (44) found that LAP has little effect upon adrenal weight in the absence of the pituitary. Stability studies in this laboratory (45) indicated that extracts of LAP have very little or no ACTH activity; it was suggested that the factor in LAP responsible for the production of pathologic lesions was not ACTH (46). The results of the present report substantiate this prediction. ACTH alone, in a dose of 1.0 U.S.P. unit thrice daily (Group 8, Experiment 6A), induced no pathologic changes in the rat. DCA-treated rats of this same experiment did ex-

hibit lesions. It may be concluded that under the conditions of these experiments, purified ACTH did not produce pathologic changes. Furthermore, the experiments reported here indicate that ACTH actually inhibits the lesion-inducing action of DCA. This is in agreement with the clinical findings of Hench *et al.* (26, 27) that ACTH as well as cortisone is of therapeutic benefit in rheumatoid arthritis and rheumatic fever. It is of interest that Selye (11, 12) now claims that growth hormone rather than ACTH is the factor in LAP responsible for inducing pathologic changes in experimental animals.

In conclusion, the GAS concept has little basis in experimental fact. Desoxycorticosterone induces pathologic changes in animals similar to those seen in the collagen diseases in man. However, this phenomenon is actually quite difficult to appraise. It has not been established that desoxycorticosterone or a related steroid is secreted by the adrenal cortex. Furthermore, the fact that sterile horse serum and certain bacteria can induce similar changes in animals weakens the strength of an argument based solely on the lesion-inducing action of desoxycorticosterone. Stress (in the non-specific sense of the word) has not been demonstrated to induce mesenchymal tissue disease in man or experimental animals. No metabolic derangement, compatible with the secretion of an excess of a "mineralocorticoid" from the adrenal cortex, has been demonstrated to occur in the collagen diseases. Rheumatoid arthritis occurs in patients with Addison's disease, a disease in which adrenocortical function is either diminished or absent. Despite recent statements to the contrary, the contents of the GAS concept did not suggest the prediction that ACTH and cortisone would be of therapeutic effect in the collagen diseases in man. The available evidence is against the notion that either hyperactivity or abnormal activity of the adrenal cortex is an etiologic factor in the mesenchymal tissue diseases in man.

Replacement therapy or pharmacotherapy. It has been demonstrated in this laboratory that DCA induces insulin hypersensitivity (22), hypernatremia and decreased brain excitability (24). These actions of DCA are counteracted by ACTH, ACE or cortisone (22, 25). The conclusion has been reached that DCA treatment leads to a state of hormone imbalance characterized by an excess of DCA (exogenous steroid) and a deficiency of endogenous 11,17-oxysteroid production. The present study indicates that still another toxic action of DCA, namely, pathologic tissue changes, can be antagonized by ACTH or cortisone. One is tempted to conclude that DCA acts to induce pathologic changes as a result of an insufficiency of cortisone-like steroids. However, in the experiments of this report, doses of ACTH and cortisone in the physiologic range, as judged by a variety of metabolic changes, failed to inhibit the development of the pathologic

changes. Large doses of these substances were required to antagonize DCA. On the other hand, physiologic doses of ACTH and cortisone will antagonize the hypernatremia and decreased brain excitability induced by DCA. It appears that the tissue changes in DCA-treated rats are not solely a manifestation of a simple deficiency of cortisone-like steroids. However, it is quite possible that such a deficiency contributes to the development of lesions. It is of interest in this connection that Criep and Mayer (47) have demonstrated that the severity of periarteritis nodosa is greater in adrenalectomized serum-treated rabbits than in serum-treated intact rabbits.

Rich and Gregory (16–18) have presented evidence in support of the concept that rheumatic-like lesions in the heart and periarteritis nodosa are pathologic manifestations of allergic phenomena. Lesions similar to those seen in the collagen diseases occur in serum-sickness in man, a disorder in which there can be no doubt of the etiologic role of an antigen. The high concentration of gamma-globulin in the serum of patients with collagen disease suggests a hypersensitivity reaction as the basis for the disorder. In rabbits injected with sterile horse serum pathologic changes develop similar to those seen in the connective tissue diseases in man. Furthermore, Rich and coworkers (48–50) have demonstrated that ACTH and cortisone can inhibit the development of periarteritis nodosa and cardiac inflammatory lesions in horse serum-treated rabbits, which is additional evidence to suggest that a close relationship exists between the etiology of the pathologic changes seen in man and in the rabbit. In the experiments reported in this paper horse serum did not produce pathologic changes in untreated or DCA-treated rats. This is surprising in view of the fact that the DCA-treated rats were hypersensitive to horse serum injection, as manifested by acute asthmatic-like reactions. There appears to be some fundamental difference between the rabbit and the rat in regard to tissue reaction to horse serum. These species differences complicate the problem in many respects. They emphasize the danger of drawing general conclusions from experiments conducted in only one species.

Since ACTH and cortisone are of therapeutic benefit in the mesenchymal diseases, the possibility may be considered that a state of absolute or relative insufficiency of the pituitary-adrenocortical system exists in these disorders. If an insufficiency exists, it cannot be the type characteristic of Addison's disease or panhypopituitarism. No metabolic derangement characteristic of adrenocortical insufficiency occurs in the collagen diseases (51); tests of the functional capacity of the pituitary-adrenocortical system reveal no significant abnormalities in these disorders (52–55). The report (56) that the rate of excretion of urinary corticoids is less than normal in patients with rheumatoid arthritis has not been confirmed (57).

The most reasonable conclusion at present is that ACTH and cortisone have a pharmacotherapeutic action in the mesenchymal diseases in man; the therapeutic dose is not an amount required to replace a deficiency, but is rather a quantity which produces titers of cortical steroids far in excess of normal tissue requirements. As in the case of DCA-induced lesions in rats, the therapeutic doses in man produce various degrees of hypercorticism.

The action of the cortical steroids in mesenchymal disease is probably one aspect of a general inhibitory effect of cortisone and related steroids upon all inflammatory processes regardless of the inciting agent, be it infectious, traumatic, chemical or antigenic [see Dougherty and Schneebely (58)]. One is not justified in concluding that the pathologic changes in the mesenchymal tissue diseases in man and in DCA-treated rats have a common etiology simply because the pathologic changes are similar and because ACTH and cortisone exert a favorable influence on both.

SUMMARY

1. Male rats were subjected to a variety of experimental conditions including DCA administration, high sodium chloride intake, unilateral nephrectomy, cold stress, and repeated injections of sterile horse serum, in an attempt to reproduce a pathologic counterpart of the collagen diseases in man.

2. DCA does not induce pathologic changes with a high degree of reproducibility from one experiment to the next. However, when pathologic changes were noted in the heart, kidney and pancreas, they were uniformly present in the DCA-treated animals of any given experiment.

3. No significant incidence of pathologic changes was encountered in rats treated with DCA and sterile horse serum despite the fact that these animals exhibited hypersenstiivity to the horse serum, manifested by an acute anaphylactic reaction.

4. Rats subjected to cold stress, DCA treatment, unilateral nephrectomy, and excess dietary sodium chloride did not exhibit an abnormal incidence of pathologic changes.

5. ACTH and cortisone, when given in large doses simultaneously with DCA, inhibited the development of the pathologic changes which occurred in animals given DCA alone. Smaller doses of ACTH and cortisone which are considered to be in the physiologic range were ineffective.

6. The mechanism by which DCA acts to induce pathologic changes similar to those seen in the collagen diseases in man is not yet established. Neither the experiments reported here nor other evidences in the literature support the concept that stress through the mediation of the adrenal cortex is an etiologic agent in the mesenchymal tissue diseases. Nor is there

evidence to support the notion that hypoactivity of the adrenal cortex is involved in the development of mesenchymal tissue disease. The most reasonable explanation for the action of ACTH and cortisone is that it is one aspect of a pharmacologic effect of cortisone, an effect characterized by inhibition of the inflammatory reaction regardless of the nature of the inciting agent.

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EFFECT OF ACTH ON THYMIC MASSES

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IN A previous paper published in 1948 (1) we reported on the effect of ACTH in a patient with myasthenia gravis and a thymic tumor. This initial study was conducted during the early days of ACTH therapy when the hormone was available in only limited amounts. The patient was treated with 40 mg. of ACTH daily for a period of four days, and treatment was followed by an appreciable decrease in the size of the thymic mass and a subsequent improvement in the myasthenic symptoms. Since this report was published, the salutary effect of ACTH on patients with myasthenia gravis without thymic enlargement has been confirmed elsewhere (2). However, Shy and his coworkers (3) have failed to observe either shrinkage of a thymic neoplasm or improvement in the symptoms of myasthenia gravis in 1 patient treated with cortisone.

The present communication is concerned with the influence of ACTH on the size of the thymic mass in 4 additional patients. Three of this group had myasthenia gravis. In 2 patients the thymic enlargement was proven to be due to tumor, which in 1 instance was malignant in character with pleural metastases. In the remaining 2 patients the thymic enlargement was demonstrated by roentgenologic study, but neither operative nor biopsy studies are available to elucidate the nature of the enlargement.

CASE REPORTS

Case 1

E. W., a 49-year-old male, was discovered to have an anterior mediastinal mass with pleural metastases on routine roentgen examination of the chest four and a half months prior to his admission to the hospital. He was symptom free and the neurologic and physical examinations showed nothing abnormal except for an increase in supracardiac dulness on percussion. Peripheral blood studies, sternal marrow aspirations, blood Wassermann and Kahn tests and urinalyses gave completely negative results. The sedimentation rate was well within the normal range. An exploratory thoracotomy, with biopsy of pleural and diaphragmatic metastatic lesions, was performed. The histologic report was "malignant thymoma."

Twenty-six days prior to the operative biopsy procedure he was treated with ACTH for a period of ten days, the daily dose being 100 mg. intramuscularly in four divided doses. This was followed by a considerable decrease in the size of the thymic mass (Figs. 1 and 2). With cessation of therapy the mass returned to its original size within two weeks

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(Fig. 3). He was again treated with 100 mg. of ACTH daily, after the biopsy, and within a period of sixteen days there once more occurred a marked decrease in the size of the thymic mass, with a suggestive shrinkage of the pleural metastatic lesions (Fig. 4).

The patient was discharged from the hospital and was followed weekly in the Out-Patient department for a period of two months. As the dosage of ACTH was gradually reduced to 40 mg. a day, the thymic mass again slowly increased in size. ACTH was inadvertently omitted for a period of seven days, and when he was seen again the follow-

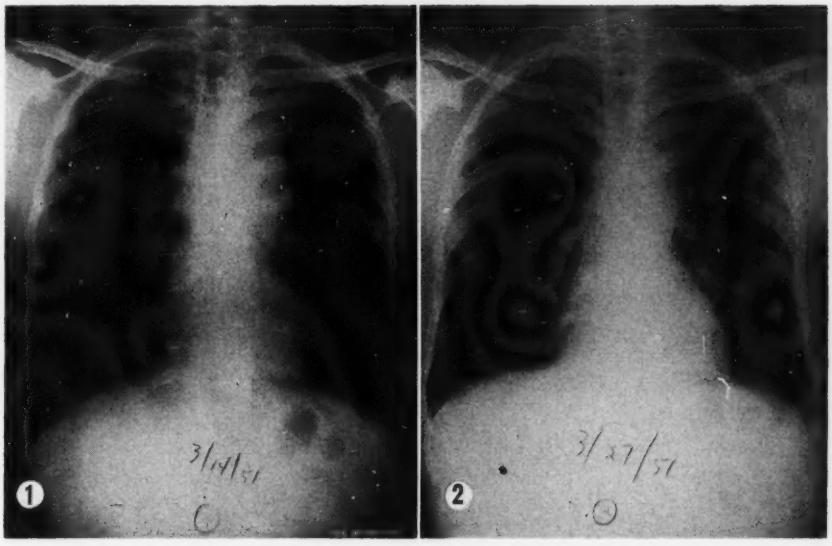

FIG. 1. *E. W.* Roentgenogram of chest prior to treatment. Note thymic mass and diaphragmatic metastasis.

FIG. 2. *E. W.* Roentgenogram of chest following completion of ten days of therapy with adrenocorticotropin. The thymic mass is considerably smaller in size.

ing week the patient complained of slight weakness. ACTH was again started in a dosage of 80 mg. a day. Three days later he was readmitted to the hospital because of severe weakness and a typical clinical picture of myasthenia gravis. Almost all of his symptoms had become manifest in the short period since his last clinic visit. The finding of a normal serum potassium level excluded the possibility of hypopotassemia. Adrenocorticotropin was stopped and prostigmine administered parenterally. It was then determined that an oral dosage of 90 to 135 mg. of prostigmine daily was adequate for control of the myasthenic symptoms. Cortisone was then given in a daily oral dosage of 200 mg. On this regimen the prostigmine requirements increased slowly over a period of three weeks to approximately 400 mg. a day. During this period the thymic mass was considerably reduced in size. Cortisone was then gradually withdrawn by decreasing the daily amount slowly over the space of a week and the prostigmine requirement fell to approximately 270 mg. a day. Following cessation of cortisone therapy the thymic mass slowly increased to its former size.

Case 2

J. M., a 5-month-old white male, was admitted to the hospital because of a six-week history of recurrent episodes of respiratory infection, otitis media, fever, and "wheezing." The physical examination revealed a febrile child with inflamed ear drums. Coarse musical rales were audible over both lung fields. Following treatment with

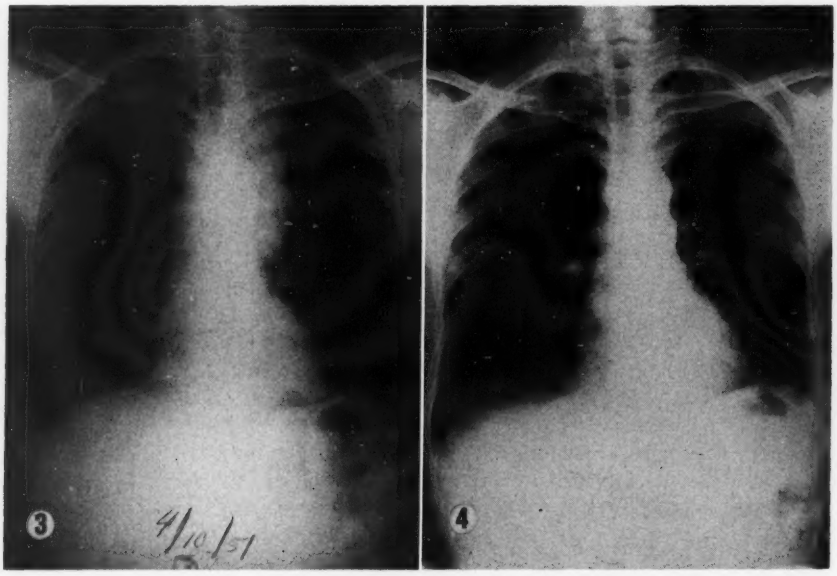

FIG. 3. *E. W.* Roentgenogram of chest two weeks following completion of first course of adrenocorticotropin. The thymic mass is now its original size.

FIG. 4. *E. W.* Roentgenogram of chest on the sixteenth day of the second course of adrenocorticotropin therapy. The thymic mass is markedly decreased in size. The diaphragmatic metastasis also appears smaller.

penicillin, chloromycetin, and moist oxygen, the signs and symptoms disappeared.

Except for a mild leukocytosis at the time of admission, the laboratory examination gave essentially negative findings. Routine roentgen examination of the chest revealed the presence of an enlarged thymic shadow.

With recovery from the infection the thymic shadow did not change significantly in size. The patient was then given daily intramuscular injections of 40 mg. of adrenocorticotropin in four divided doses for a period of nine days. In spite of the difficulties attendant on obtaining comparable roentgenograms in an infant, the size of the mass appeared to shrink significantly. The right margin of the thymus, which protruded well beyond the cardiac border on repeated examinations made prior to therapy, extended only to the right border of the heart following completion of the course of treatment (Figs. 5 and 6).

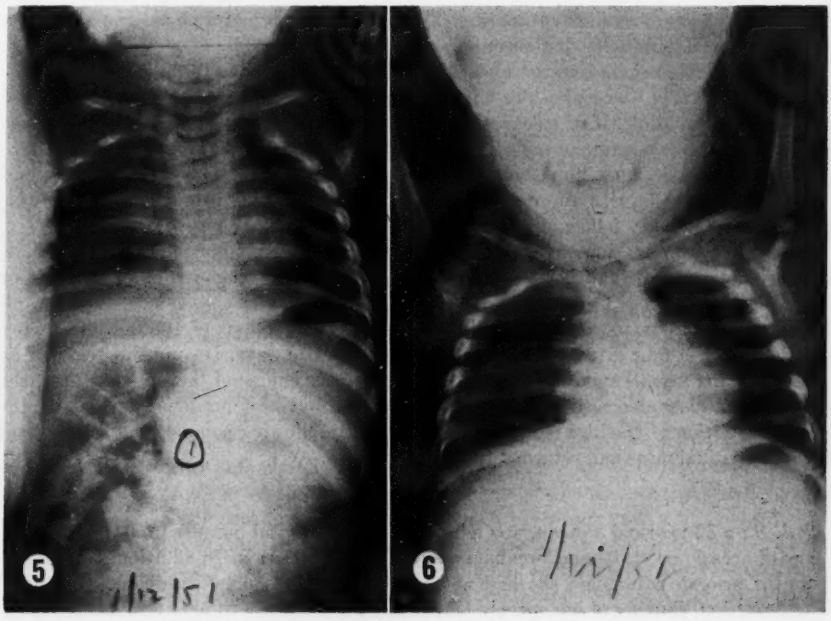

FIG. 5. *J. M.* Roentgenogram of chest prior to treatment with adrenocorticotropin.
FIG. 6. *J. M.* Roentgenogram of chest on the tenth day of treatment with adrenocorticotropin.

Case 3

P. M., a 40-year-old white woman, was admitted to the hospital for the first time in July, 1950, complaining of weakness, diarrhea, irritability, heat intolerance, shaking of the hands, polyphagia, dysphagia, the development of a stare, and weight loss of six months' duration.

The physical examination revealed an enlarged thyroid over which a bruit was audible, a moist skin, and a marked tremor of the tongue and hands. There was a stare and a mild bilateral lidlag. The liver was enlarged to two finger breadths below the costal margin, and there was some edema of the lower extremities. The findings on neurologic examination were essentially negative.

The laboratory examination revealed a normal urine and blood count. The blood cholesterol level was 240 mg. per cent. The basal metabolic rate was +36 and +31 per cent on two occasions. The urinary excretion of a tracer dose of I^{131} was 5 per cent. The arm-to-tongue circulation time was rapid, being seven seconds. The Jolly test and exercise test for myasthenia gravis gave negative results.

The diagnosis of hyperthyroidism was made. The patient was treated with 5 millicuries of I^{131} and discharged for further observation in the clinic.

She was readmitted to the hospital six months later. There had been marked improvement in the stare, lidlag, sweating, heat tolerance, and diarrhea. However, her generalized weakness and dysphagia had become worse, and in addition she had noted

tremor of the hands and incoordination. There was no history of diplopia nor of increasing excessive fatigue following exertion.

The physical examination revealed ptosis of the right eyelid and slight exophthalmos of the left eye. The external ocular muscles were normal. The thyroid was enlarged, but no bruit was audible. The spleen tip was just palpable. The findings on neurologic examination were negative except for the rapid development of muscle fatigability.

The neurologic consultant concurred in the diagnosis of myasthenia gravis and further confirmation was afforded by the disappearance of the ptosis and the decrease in muscle fatigability following parenteral administration of 1.5 mg. of prostigmine.

Roentgen examination of the chest demonstrated a widening of the superior mediastinal shadow predominantly to the right, consistent with a diagnosis of an enlarged or persistent thymus.

The patient was treated with daily intramuscular injections of 100 mg. of adrenocorticotropin in four divided doses for a period of fifteen days. During this regimen no change in the myasthenia was observed. Following the course of therapy, however, the mediastinal mass decreased in size. This change could best be demonstrated by the superimposition of tracings of the mass. One month following completion of treatment the mediastinal mass had returned to its original size.

Case 4

B. S., a 32-year-old woman, was admitted to the hospital because of a two-year history of occasional stuttering, weakness, and blurring of vision. In addition, for the two months prior to admission she had noted ptosis of the eyelids, dysphagia, and nasal regurgitation, slurring of her speech, and increasing weakness of her arms and legs.

The physical examination revealed nothing abnormal, except for diminished motor power in all four extremities, ptosis of the eyelids, and a nasal quality of speech.

The laboratory examination revealed a normal urine and blood count. Roentgen examination of the chest with the aid of laminography revealed a small round mass, about 1½ inches (3.8 cm.) in diameter, in the anterior mediastinum.

The diagnosis of myasthenia gravis was made and confirmed by demonstration of a marked improvement in the signs and symptoms following parenteral administration of 1 mg. of prostigmine.

After a period of observation as to the prostigmine requirements, she was given 100 mg. of adrenocorticotropin a day by intramuscular injection in four divided doses for four days. Following the onset of this therapy the myasthenic symptoms became markedly aggravated and the patient lapsed into a myasthenic crisis, requiring frequent large doses of prostigmine. In addition, she was given potassium salts to correct any depletion of the stores of this ion as a result of adrenocorticotropin therapy. Following cessation of treatment, the requirements for prostigmine gradually decreased to the previous level. On two occasions during the course of adrenocorticotropin therapy tomographic sections failed to demonstrate the mediastinal mass as clearly as it had been seen either prior to treatment or after the therapy had been discontinued.

The patient was operated upon sixteen days after completion of the course of treatment with ACTH. A firm globular mass 4 cm. in diameter and weighing 55 Gm. was removed from the superior mediastinum. On section the capsule was thick and fibrous, being 2–3 mm. in depth. The pathologic report was "Thymoma with the structure of hyperplastic thymus (epithelial) tissue."

The postoperative course was uneventful and she was discharged approximately five weeks after operation. The postoperative prostigmine requirements were essentially the

same as during the control period of observation prior to the course of adrenocorticotropin therapy.

Case 5

T. N. (previously reported (1)), a 29-year-old white female, had noted drooping of the eyelids, difficulty in speaking, and inability to raise the arms for a year prior to admission. She had consulted a physician, who gave her an injection of prostigmine with striking relief of the symptoms. She then was given increasing amounts of oral prostigmine, as much as 300 mg. daily, for approximately four months prior to entrance into the hospital.

The physical examination revealed nothing abnormal. There were no muscular fibrillations or atrophy. The thyroid was not enlarged. Roentgenographic studies of the chest revealed the presence of a mass in the anterior mediastinum, extending to the left between the second and fourth ribs. Urine and blood count were normal. The basal metabolic rate was +6 per cent. During her hospital stay the diagnosis of myasthenia gravis was confirmed by neurologic investigation.

The patient was then given 40 mg. of adrenocorticotropic hormone daily by intramuscular injection in four equally divided doses for four days. During the period of treatment with adrenocorticotropic hormone there was no change in either the patient's symptoms or the daily prostigmine requirements. Three or four days after therapy was discontinued, however, the patient began to experience an increase in the sense of well being and an increase in strength. The daily need for prostigmine became progressively less, and starting thirty-two days after completion of therapy with adrenocorticotropic hormone she required no prostigmine at all.

After four days of treatment with adrenocorticotropic hormone a roentgenogram of the chest showed a marked decrease in the size of the mediastinal mass as compared with all previous roentgenologic examinations. The mass remained considerably reduced in size for two weeks after cessation of therapy and then began to increase. By the end of the third week after discontinuation of therapy the mass had attained its original size.

SUMMARY

Five patients with enlargement of the thymus were treated with intramuscular ACTH for periods which varied from four to sixteen days. In 2 of these instances the thymic enlargement was proven to be due to tumor, 1 of which was malignant in character with proven metastases. In the remaining 3 patients the precise nature of the enlarged thymus was not established. In the case of the malignant thymoma (Case 1) the decrease in size of the mass as the result of treatment with ACTH or cortisone was striking. In addition, the roentgenologic studies suggested temporary shrinkage of the pleural metastatic lesions following treatment with ACTH. In 1 patient with thymic enlargement, the character of which was undetermined (Case 5), there occurred an almost equally marked decrease in size of the thymic mass after four days of treatment with ACTH. In the remaining 3 patients the shrinkage of the thymic lesion appeared to be significant, although not as striking as in the foregoing 2 cases.

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SOME EFFECTS OF ADRENOCORTICOTROPIC HORMONE IN VARIOUS HYPO-OVARIAN STATES*

I. SECONDARY AMENORRHEA

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FAILURE of normal ovarian function with the resultant cessation of the menses in an otherwise healthy, normal young woman may be due to a variety of causes. Most often it is an expression of a disturbance of the hormonal support necessary for normal cyclical activity. The failure may involve the ovary primarily, as in the menopausal period. It may be the result of malfunction of the gonadotropic activity of the anterior lobe of the hypophysis. It may possibly involve other endocrine glands such as the thyroid or adrenal, which support the hormonal balance necessary for the normal sex cycle.

The role of the adrenal gland in ovarian function is not known. There is some evidence that adrenocortical activity is important in both normal and abnormal ovarian function. The present investigation was undertaken in order to study mild and severe ovarian failure in women in whom ovarian function had been established, and to demonstrate the metabolic effects of ACTH and its influence on disturbed ovarian activity.

It was not intended to demonstrate any therapeutic effect of ACTH, and the results in no way indicate that ACTH is to be recommended for the treatment of secondary amenorrhea.

Certain observations made by others with regard to the effects of ACTH in women are pertinent. Forsham *et al.* (1) in 1948 stated that 3 female patients who had not menstruated previously for at least one year, one of whom had Addison's disease, noted menstrual flow six to ten days after the injection of a single dose of 25 mg. of ACTH. Conn (2) in the same year reported that 2 normal female subjects experienced gross irregularity of the menstrual cycle following the injection of 50 mg. of ACTH daily for

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eight days. Later Sprague *et al.* (3) reported that in a 49-year-old woman whose menstrual periods had been irregular for one year, 100 mg. of ACTH given daily for twelve days increased biologic urinary estrogenic activity from 0 to 230 rat units per twenty-four hours. Farnsworth (4) reported the temporary re-establishment of regular menses in a 23-year-old amenorrheic girl with nephrosis, following treatment with ACTH.

We administered ACTH to 21 women in all. This paper will describe the results in 13 patients. Of these, 12 had secondary amenorrhea, and 1 had excessive uterine bleeding. The other 8 patients—2 with primary amenorrhea, 2 menopausal women, and 4 castrates—will be discussed in a subsequent communication, as a separate group. Table 1 gives the scheme of numbering of the patients, as well as certain essential clinical data, for the first 13 cases studied.

TABLE 1. CLINICAL DATA AND DOSAGE OF ACTH

Case	Age (yrs.)	Diagnosis	ACTH			Days from end of treatment to end of bleeding	Days from last spontaneous menstrual period to beginning of treatment with ACTH
			No. of days administered	Total amount (Gm.)	Average daily dose (mg.)		
1. (R.B.)	31	Secondary amenorrhea, infertility	6	0.300	50	36, 15	270
2. (S.S.)	28	Secondary amenorrhea, obesity, hirsutism	6	0.300	50	100	18
3. (K.W.)	34	Secondary amenorrhea, infertility	6	0.600	100	90	90
4. (T.J.)	38	Excessive uterine bleeding, infertility	6	0.600	100	13	15
5. (V.R.)	27	Secondary amenorrhea	8	0.800	100	16	30
6. (M.B.)	21	Secondary amenorrhea	8	0.800	100	No bleeding	365
7. (S.F.)	26	Secondary amenorrhea	8	0.800	100	2	60
8. (K.H.)	31	Secondary amenorrhea	10	1.000	100	2	13
9. (E.M.)	24	Secondary amenorrhea (post partum)	10	1.000	100	170	1,095
10. (B.W.)	25	Secondary amenorrhea	8	0.800	100	No bleeding	1,095
11. (J.G.)	30	Secondary amenorrhea, anorexia nervosa	11	0.575	52.27	No bleeding	17
12. (E.D.)	22	Secondary amenorrhea, eunuchoid body configuration	8	0.800	100	No bleeding	730
13. (B.G.)	29	Secondary amenorrhea	6	0.600	100	5	334

METHODS

Except where noted, the subjects were all given weighed diets containing the same food each day, of sufficient caloric content to maintain weight, with activity in the hospital in accordance with the particular individual's desires. The food was not analyzed, but a fairly constant intake was attained by serving all meat from a single beef round throughout each experiment, by serving the cooked foods salt-free and adding measured amounts of salt at the table, and by using largely canned fruits and vegetables. Each patient ate all of her food each day, almost without excep-

tion. Six to eight days of the constant diet preceded treatment with ACTH; a longer control period would have been desirable, but could not be attained.

Urines were collected over 24-hour periods, without added preservatives, and were kept in a small ice box in the patient's room during the collection period. All collections were checked for completeness by creatinine determinations.

All chemical determinations were performed in duplicate, except the chemical assays for urinary corticoids (neutral reducing lipids). Total nitrogen in the urine was determined by the micro Kjeldahl method, distilling the ammonia formed into boric acid solution, according to the procedure described by Ma and Zuazaga, and titrating to the brom cresol green endpoint (5). Urine inorganic phosphorus was determined by the method of Fiske and SubbaRow (6); chloride by the Volhard-Harvey method (7); and sodium and potassium directly with a Beckman flame photometer. Crude neutral 17-ketosteroids were determined by a modification of the method of Holtorff and Koch (8), using dehydroisoandrosterone acetate as a standard with values expressed in terms of dehydroisoandrosterone; and urinary corticosteroids (neutral reducing lipids) by the method of Corcoran and Page (9), in which the side chains of the C21 steroids are oxidized with periodic acid, followed by colorimetric measurement of the formaldehyde formed in the oxidation. Pregnanediol was determined by the method of Guterman as modified by Davis and Fugo (10), which detects with reasonable accuracy 2 mg. per liter of urine. Urinary gonadotropins were assayed on 21-day-old female rats by the method of Witschi, after precipitation with 4 volumes of 95 per cent alcohol (11). Eosinophil counts were uniformly made four hours after the initial morning dose of ACTH, which was given at 6:00 a.m. Determinations of the serum sodium, potassium, chloride, pH and CO_2 were made before treatment was given and were repeated at the end of the treatment period. Similarly, electrocardiograms were taken before and at the end of the treatment period.

RESULTS

Of these 13 patients, 9 had episodes of uterine bleeding following treatment with ACTH, as shown in Table 1. In none of these 9 was ovarian failure severe or complete, as judged by the appearance of the vaginal mucosa and external genitalia, and the size of the uterus. In 6 (Cases 2, 3, 4, 5, 7, and 8) of the 9 patients spontaneous uterine bleeding had occurred within six months prior to therapy with ACTH; hence, the episodes of uterine bleeding which occurred after therapy may have been spontaneous in nature. In Case 1 and in Case 13 it seems logical to attribute the menstrual periods following ACTH to the treatment; detailed protocols for these patients follow.

Case 1. R. B. was a 31-year-old-white married woman whose menses had become

progressively scantier in amount and less frequent for three years. During this period she gained 25 pounds in weight. She had never had normal monthly cycles; at most she menstruated seven times a year. Menarche was at the age of 17–18 years. There was no family history of menstrual irregularity or failure of sexual development. The last menstrual period was in October 1949, lasting ten days, with heavy flow and preceded by abdominal cramps. The previous menstrual period was a scanty one in May 1948, lasting only three days. She had never been pregnant, although contraception was not practiced. Physical examination showed nothing abnormal except for a masculine-type distribution of pubic hair. Her height was 156 cm. and weight, 61.3 Kg. Pelvic examination gave normal findings except that in the right adnexal region there was a small

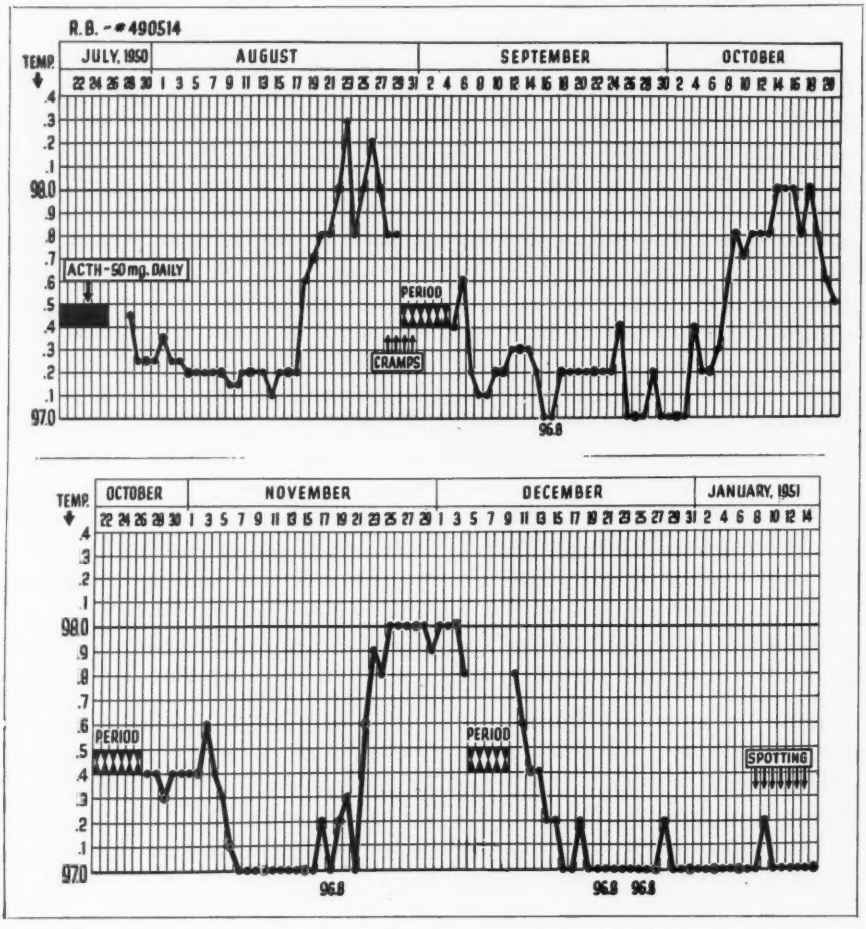

FIG. 1. Graphic record of basal body temperature, showing periods of menstrual bleeding in Case 1, R. B., aged 31, secondary amenorrhea.

(approx. 4×4 cm.) mass, freely movable and not tender, and second-degree retroversion of the uterus. A roentgenogram showed a normal sella turcica. The basal metabolic rate was −10 per cent (Mayo Foundation standards). Routine laboratory examination showed the blood and urine to be normal. Vaginal smears resembled those seen in the early pre-ovulatory phase of a normal menstrual cycle.

The patient was treated for six days with ACTH (Armour) given intramuscularly at 6-hour intervals in doses[1] of 50 mg. daily from July 20 through July 25, 1950. Subsequently normal menstrual periods occurred as follows: August 30 through September 4,

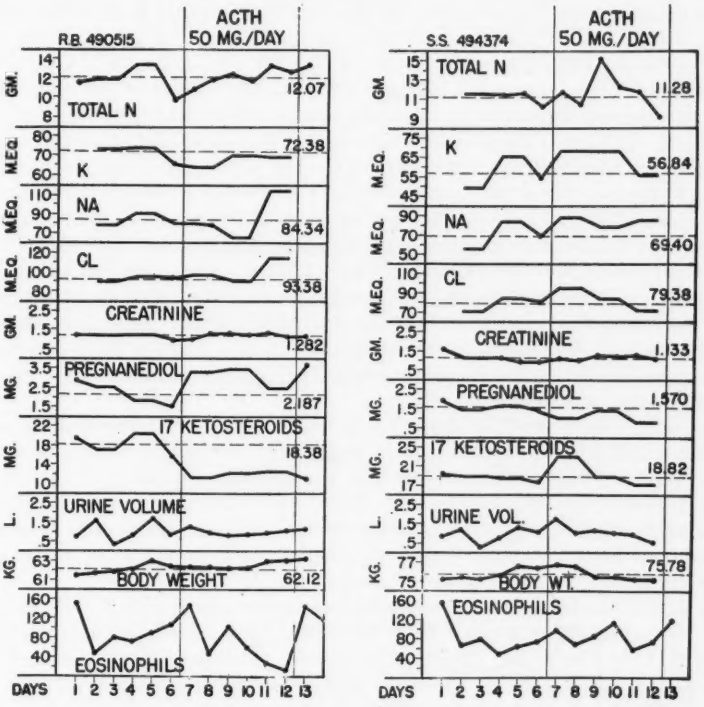

FIG. 2. The effects of ACTH on several urinary constituents and on total circulating eosinophils and body weight of R. B. (Case 1), aged 31, secondary amenorrhea, and S. S. (Case 2), aged 28, secondary amenorrhea, obesity, and hirsutism. The horizontal interrupted lines and the corresponding figures to the right mark pre-treatment baselines. Isolated dots mark individual daily determinations; otherwise, two-day pools provide the information expressed by running lines.

1950; October 21 through 26, and December 5 through 9. The period of August 30–September 4 was accompanied by lower abdominal cramping pains on the first two days. Each period was preceded by a significant rise in the basal body temperature (Fig. 1).

[1] Dosages noted in this case and subsequent ones are in terms of Armour standard LA 1 A.

Slight blood spotting occurred during the period January 8 through 14, 1951, but this was not preceded by a rise in the basal body temperature. Spotting occurred again on January 31 through February 2, but thereafter all vaginal bleeding ceased. An endometrial biopsy specimen taken February 1, 1951, showed normal proliferative endometrium. A second course of ACTH (Armour), in a dosage of 50 mg. daily, was given April 10 through 15, 1951. This was again followed by a normal menstrual period on April 25 through 30, accompanied by abdominal cramps on the first two days of the period. This period also was preceded by a rise in the basal body temperature (not shown in Fig. 1).

The data shown in Figure 2 were obtained at the time of the first ACTH treatment period in July, 1950. The patient was on a constant diet of C 157, P 81, F 126, calories 2,086, containing an estimated 12.96 Gm. of N, 2.273 Gm. of Na, 1.817 Gm. of Cl and 3.372 Gm. of K. She received in addition 3 Gm. of NaCl added at the table. Distilled drinking water was allowed ad lib.; the daily intake varied from 520 to 1,040 cc.

Case 13. B. G. was a 29-year-old white married woman whose last normal menstrual period was in May 1950. Menarche was at the age of 10½ years, and the menses were regular and normal thereafter. In 1948 she had a spontaneous abortion at four and a half months' gestation, and in June 1949 gave birth to stillborn twins at seven and a half months. The menses were irregular after this, and from October 1949 to March 1950 there was no menstrual bleeding at all. The patient was given desiccated thyroid, 0.06 Gm. daily, in November 1950 and this dosage was continued throughout the period of metabolic study. She was 159 cm. tall and weighed 61.6 Kg. The findings on physical examination, including the pelvis, were essentially normal. Routine laboratory examination showed the blood and urine to be normal. The basal metabolic rate was −9 per cent (Mayo Foundation standards).

The patient was treated for six days with ACTH (Armour) given intramuscularly at 6-hour intervals in doses of 100 mg. daily from April 17 through 22, 1951. A normal menstrual period followed treatment—April 27 through May 3, 1951, and another period occurred May 26 through May 28, 1951. The course in the hospital was complicated by a right pyelonephritis, which developed on April 20, 1951. A nonhemolytic streptococcus was cultured from the urine. The symptoms subsided promptly on treatment with Chloramphenicol.

The data shown in Figure 3 were obtained during treatment with ACTH. The patient was on a constant diet of C 186, P 86, F 59, calories 1,619, containing an estimated 13.77 Gm. of N, 1.480 Gm. of Na, 2.588 Gm. of Cl and 2.653 Gm. of K. She received in addition 3 Gm. of NaCl added at the table. Distilled drinking water was allowed ad lib.; the daily fluid intake varied from 1,120 to 2,660 cc.

In 3 of the 4 patients who had no bleeding after ACTH therapy, the degree of ovarian failure was in all probability more severe. Thus, M. B. (Case 6) had had no menstrual bleeding for three years; B. W. (Case 10) had not menstruated for three years and the uterus was small; E. D. (Case 12) had not menstruated for two years, the external genitalia were immature, and the uterus was small. J. G. (Case 11) did not bleed after ACTH, although she had a scanty spontaneous menstrual flow sixteen days before treatment was started. She was the only patient in the entire series in poor general physical condition.

In general, changes in urinary excretion of the various metabolites stud-

ied follow the patterns which have been described by others (3, 14, 15, 16). Nitrogen excretion increased in most of the patients, although the changes were small in magnitude when compared to those seen after the

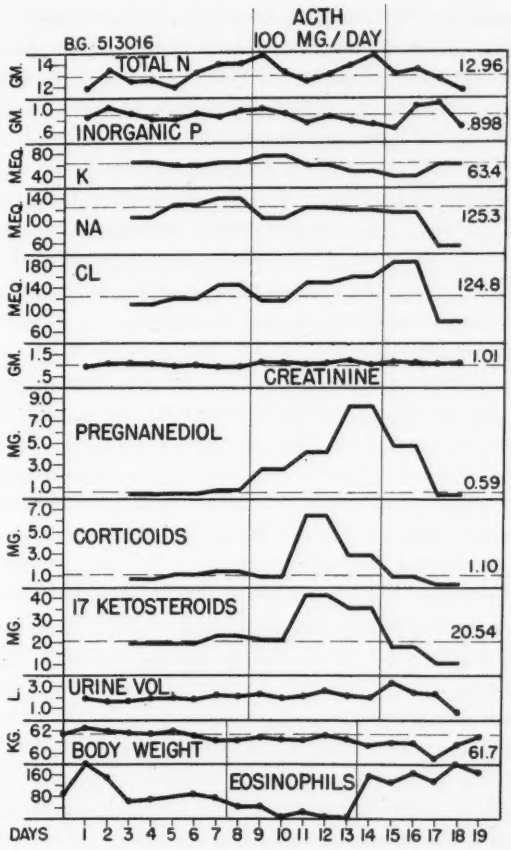

FIG. 3. The effects of ACTH on several urinary constituents and on total circulating eosinophils and body weight of B. G. (Case 13), aged 29, secondary amenorrhea. The horizontal interrupted lines and the corresponding figures to the right mark pre-treatment baselines. Isolated dots mark individual daily determinations; otherwise, two-day pools provide the information expressed by running lines.

administration of estradiol or testosterone (17). Urinary inorganic phosphorus decreased in all cases in which it was determined, with one exception—Case 10. This retention of phosphorus is in agreement with the results obtained by Albright's group in panhypopituitarism (18) and stands

in contrast to the changes in phosphorus excretion reported by Thorn (16) and by Sprague (3), although the normal man (R. P.) studied by Thorn did show a slightly diminished phosphorus excretion during ACTH therapy. The recovery periods in our own study were shorter than is customary in metabolic experiments, but for the most part the changes in nitrogen and phosphorus excretion observed during the administration of ACTH were promptly reversed at the cessation of treatment.

Similarly, the changes in urinary excretion of potassium, sodium, and chloride were much the same as those observed by Albright's group. Thus, in nearly every case there was an initial increase in potassium excretion, followed by a decrease. Sodium was retained early in the treatment period, but the urinary sodium output rose later, exceeding the average control value in 7 of the 13 patients. Chloride excretion paralleled that of sodium almost invariably. There was no significant change in the serum levels of sodium, potassium or chloride during therapy.

The response to treatment with ACTH, as shown by urinary 17-ketosteroid excretion, was in general that which has been reported by others (1, 3, 14, 15). We do not attach significance to the apparent fall in urinary 17-ketosteroids during ACTH therapy in Case 1 (Fig. 2). The slight rise on days 7 and 8 in Case 2 is also probably of no significance. Mason *et al.* (14) found no increase in urinary 17-ketosteroids, using daily 50-mg. doses of ACTH in a 32-year-old normal woman—the same dose which we employed in the treatment of Cases 1 and 2.

Significant increases in urinary pregnanediol excretion were observed in 10 of the 13 patients during administration of ACTH. The patterns of excretion were not consistent from one patient to another, however. For example, in Cases 6, 7, 8, 9, 10 and 12 the increase was not progressive, and sharp falls in urinary pregnanediol occurred prior to the cessation of ACTH therapy. The maximum amount excreted was 13 mg. per twenty-four hours, in Case 13. It would perhaps be more fitting to use the term "pregnanediol complex" with reference to the steroid material determined in this study. Final chemical identification of the product which gave color with concentrated sulfuric acid was not carried out. Sommerville (19) has warned that other steroids may be determined by this method when the urine contains large amounts of other neutral steroids of adrenal origin. Thus, androsterone, isoandrosterone, and pregnanolone are not completely removed in the process of purification of the chromogenic material. Under ordinary circumstances they are much weaker chromogens than pregnanediol, but if present in large amounts, as might be the case when the adrenal is stimulated with ACTH, they may contribute significantly to the color produced on addition of concentrated sulfuric acid. Dehydroisoandrosterone is a powerful chromogen, but is usually completely elimi-

nated by the precipitation process. Landau (20) has recently shown that the administration of ACTH in man markedly increases the urinary excretion of dehydroisoandrosterone. We, therefore, tested our pregnanediol method with dehydroisoandrosterone and found that this compound was completely eliminated by the time the point of color development was reached. Nevertheless, we hesitate to make conclusive statements with regard to the effect of ACTH on urinary pregnanediol until the product has been finally purified and chemically characterized.

Since it is generally accepted that pregnanediol is one of the chief end-products of progesterone metabolism, and since the administration of progesterone to patients with secondary amenorrhea is often followed by uterine bleeding, the source of the increased urinary pregnanediol in our patients becomes a matter of considerable interest. Progesterone has been isolated from both corpus luteum and the adrenal gland (21). In the cases here reported, the increased urinary pregnanediol could therefore represent an increase in progesterone production by either of these glands, although the adrenal cortex would logically be the first choice, in view of the nature of the stimulating agent (ACTH). In a subsequent communication, we shall report on the effect of ACTH in castrated women, in order to elucidate this point.

SUMMARY

1. Short courses (6–10 days) of treatment with ACTH have been given to 12 young women with secondary amenorrhea and to 1 woman with excessive uterine bleeding. The excretion of various urinary metabolites and steroid hormones has been followed.

2. Uterine bleeding followed therapy in 9 of the 13 patients, but probably in only 2 of these should the bleeding be attributed to treatment with ACTH. In none of the patients showing clinical evidence of marked ovarian failure was there bleeding following treatment with ACTH.

3. In 10 of the 13 patients crude urinary pregnanediol was increased during treatment with ACTH, reaching levels as high as 13 mg. per twenty-four hours in one case.

4. Metabolic effects, as judged by the urinary excretion of nitrogen, phosphorus, potassium, sodium and chloride were similar to those reported by others.

Acknowledgments

The authors are indebted to Dr. Richard Landau and Miss Ruth Loughead for expert technical assistance; to Mrs. Regina Malloy and Mrs. W. Stateson of the nursing staff, and to the members of the house staff of the Chicago Lying-in Hospital, who assisted in this study.

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THE INFLUENCE OF ACTH ON THE SODIUM AND POTASSIUM CONCENTRATION OF HUMAN MIXED SALIVA

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RECENT studies indicate that ACTH can stimulate the salt-regulating function of the adrenal cortex in humans, thereby decreasing the excretion of sodium and increasing that of potassium in the urine (1, 2, 3), and decreasing the sodium concentration of sweat (3, 4). However, there are certain technical difficulties involved in the collection of suitable specimens of these fluids for this type of investigation. Hence, the influence of ACTH on sodium and potassium concentration was investigated in saliva because of its easy availability. This paper will indicate that the use of saliva has other advantages.

METHODS

Four patients receiving ACTH[1] for therapeutic reasons were investigated; 3 were psychoneurotic (2 women, J.L. and K.H.; and 1 man, G.M.)[2] and the fourth had emphysema (a male, K.R.). ACTH was administered intramuscularly every four to six hours (see Figures 1, 2, 3, and 4 for dosage levels), and placebo injections were administered for several days after treatment.[3] The sodium and potassium intake of these patients was not controlled throughout the period of study; only K.R.'s diet deviated from the routine hospital fare, in that it had a fixed protein (100 grams) and caloric (2,700 calories) content.

A 4-cc. sample of unstimulated mixed saliva was collected from each patient daily before each meal and at bedtime for several days before, during, and after ACTH. Each sample was centrifuged and aliquots of the supernatant fluid were withdrawn and diluted, 1:25. Sodium and potassium were then determined by a Perkin Elmer flame photometer (5). The Na and K concentration values of the saliva specimens and the ratio of

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[1] Armour's.

[2] I am indebted to Dr. D. Ewen Cameron, director of the Allan Memorial Institute of Psychiatry, for permission to investigate patients G.M., K.H., and J.L., and to Dr. Bram Rose of the Royal Victoria Hospital, for patient K.R.

[3] Each placebo injection consisted of the solvent in which ACTH was administered, that is, 3 cc. of a solution containing 2.5 cc. of sterile physiologic saline or distilled water, and 0.5 cc. of novocaine.

their concentrations, Na/K, were plotted on the same graph, a separate graph being made for each series of samples collected at the same time of day (Figs. 1, 2, 3 and 4). The Na/K values were also plotted in the order in which the specimens of saliva were collected, that is, the "before breakfast" value first, the "before lunch" value second, etc. (Fig. 5).

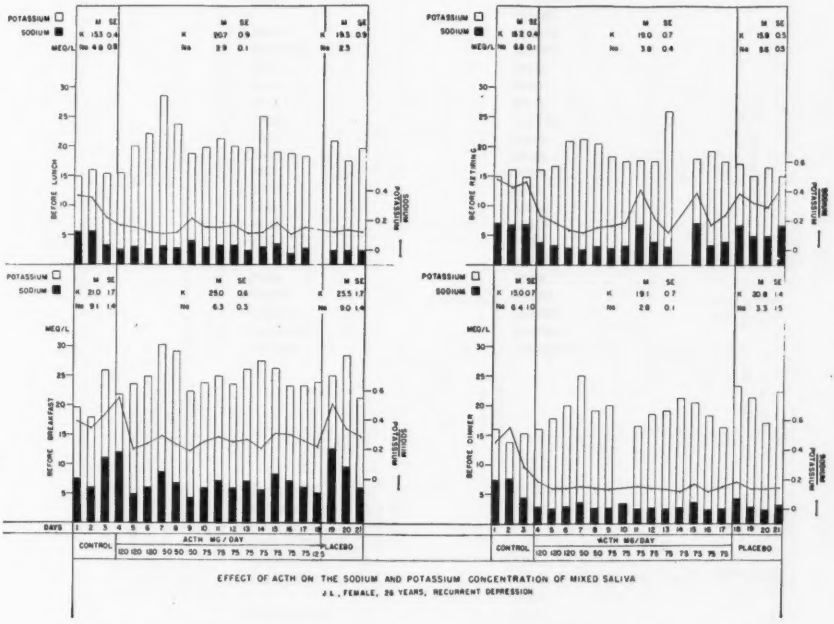

FIG. 1. ACTH injections were begun after breakfast on Day 4, and stopped after lunch on Day 18.

In 1 patient (K.R., Fig. 4), the daily urinary excretion of the 17-ketosteroids and the daily counts of the circulating polymorphonuclear eosinophils were also determined (Fig. 4).[4]

The results were examined statistically by "t" and χ^2 tests (8) and if the probability of a difference was less than 0.05, the results were considered significant. Statistical comparisons were made between a) values of samples collected during ACTH therapy and the values of those procured prior to treatment, and between b) the ACTH samples and those

[4] My thanks are due to Drs. J. S. L. Browne and Bram Rose of the Royal Victoria Hospital, in whose laboratory the determinations of the 17-ketosteroids (6) and circulating eosinophils (7) were made.

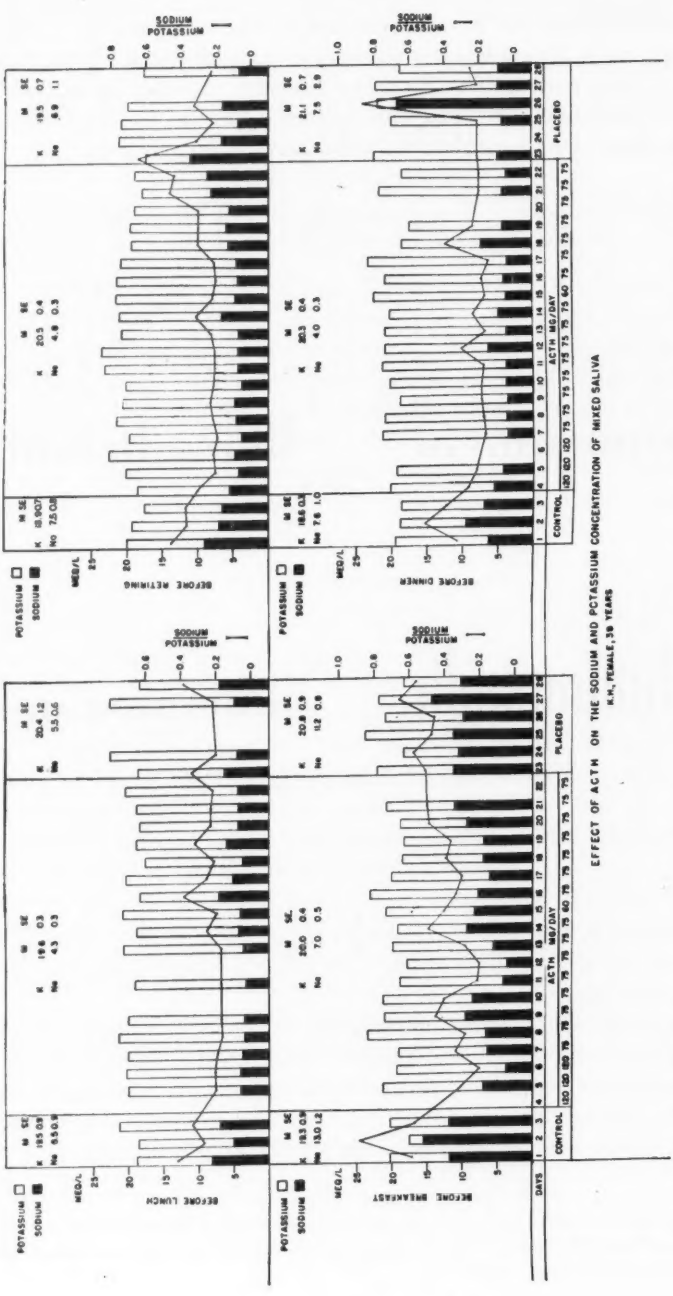

FIG. 2. ACTH therapy was instituted after breakfast on Day 4, and withdrawn before retiring on Day 22.

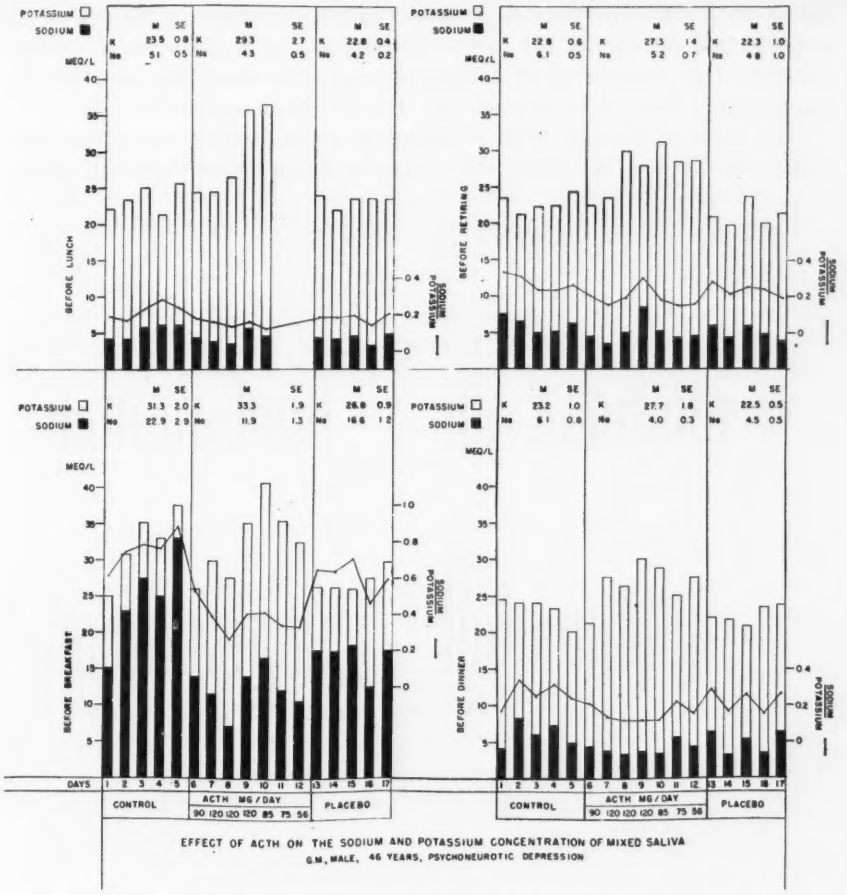

FIG. 3. Treatment with ACTH began after breakfast on Day 6, and ceased before retiring on Day 12.

collected after treatment was stopped. The former comparison tested the effect of administering the hormone; the latter, the effect of withdrawing it.

RESULTS

Figures 1, 2, 3 and 4 reveal that upon administration of ACTH the mean sodium concentration of the saliva decreased in all of the 16 experiments, 12 times in a statistically significant manner. Upon withdrawal of the hormone the mean Na concentration rose again in 13 out of 16 experiments, 5 times significantly.

Simultaneously with the above changes in the Na concentration, the

mean potassium concentration of the saliva increased in 13 out of 16 experiments during ACTH treatment, 8 times in a statistically reliable manner. Upon cessation of ACTH therapy, the mean K concentration decreased in 9 out of 16 experiments, 4 times significantly.

The value of the Na/K ratio decreased in each of the 16 experiments when ACTH was instituted, the decrease being statistically significant

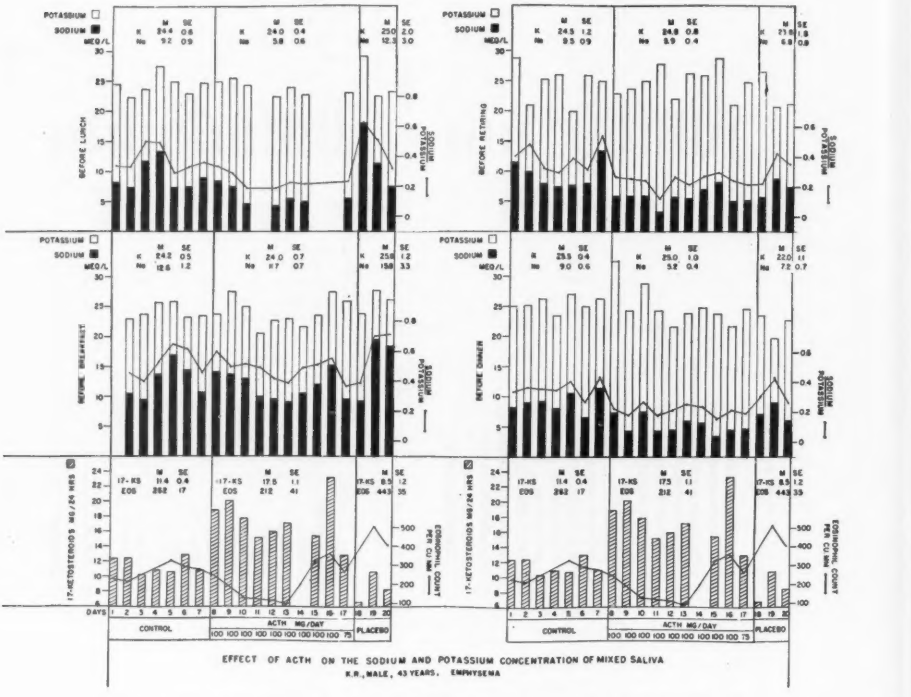

FIG. 4. The administration of ACTH was instituted after breakfast on Day 8, and stopped after dinner on Day 17.

15 times. When ACTH was stopped, the value of the Na/K ratio rose again 15 out of 16 times, 6 times significantly.

The highest daily value in the Na/K ratio occurred before breakfast in each of the 4 patients throughout the entire period of investigation (Fig. 5). The administration of ACTH did not alter the salivary Na/K diurnal rhythm, although the cycle occurred at a lower level. The daily variation in the Na/K ratio is due chiefly to the variations in the Na concentration (Figs. 1 to 4). Measurements made in this laboratory on 35 other subjects confirmed this finding (9).

There was a significant rise in the urinary 17-ketosteroids of K.R. during ACTH treatment as compared with the pre-treatment controls ($P<0.001$), and a significant fall upon withdrawal of the hormone ($P<0.001$) (Fig. 4).

The number of circulating eosinophils fell significantly on Days 10, 11, 12, and 13 as compared with the pre-treatment counts ($0.05>P>0.02$). The post-treatment eosinophil count was also significantly greater than that obtained during ACTH therapy ($P<0.001$).

DISCUSSION

These data indicate that ACTH can significantly reduce the concentration of sodium in saliva while increasing that of potassium. Moreover, these changes cannot be entirely secondary to changes in the water content of this fluid, because in our experiments the Na/K ratio of the saliva also decreased during ACTH therapy (Fig. 5). Furthermore, a fall in the concentration of Na and a simultaneous rise in that of K, consequent to ACTH therapy, were frequently observed in the same sample of saliva, *e.g.*, in the saliva samples collected from J.L. before breakfast, before dinner and before retiring on Days 5 and 6 (Fig. 1), in the "before retiring" samples collected from K.H. on Day 6 (Fig. 2), in the "before dinner" samples collected from G.M. on Day 10 (Fig. 3), and in the "before retiring" sample collected from K.R. on Day 11 (Fig. 4).

To determine whether ACTH in the present study caused a net Na retention and K excretion in the saliva would require information about the changes in the rate of flow of the saliva during treatment, in addition to the changes in the Na and K concentration already described. Although the salivary rate of flow was not investigated in the present study, several conclusions can be drawn: a) the decrease in the $\frac{\text{Na concentration}}{\text{K concentration}}$ due to ACTH indicates a decrease in the $\frac{\text{Na amount}}{\text{K amount}}$ regardless of how the salivary rate of flow was influenced by ACTH; b) even if ACTH changes the rate of flow of the saliva, then either the Na *retention* or the K *excretion* was greater than that indicated by the changes in the Na or K *concentration*, depending on whether the rate of flow was decreased or increased; c) only if the rate of the salivary flow remains unchanged during ACTH therapy does the decrease in the Na *concentration* and the increase in the K *concentration* in the saliva indicate a decrease in the *amount* of Na and simultaneously an increase in the *amount* of K.

The effect of ACTH on the Na/K ratio of the saliva was probably medi-

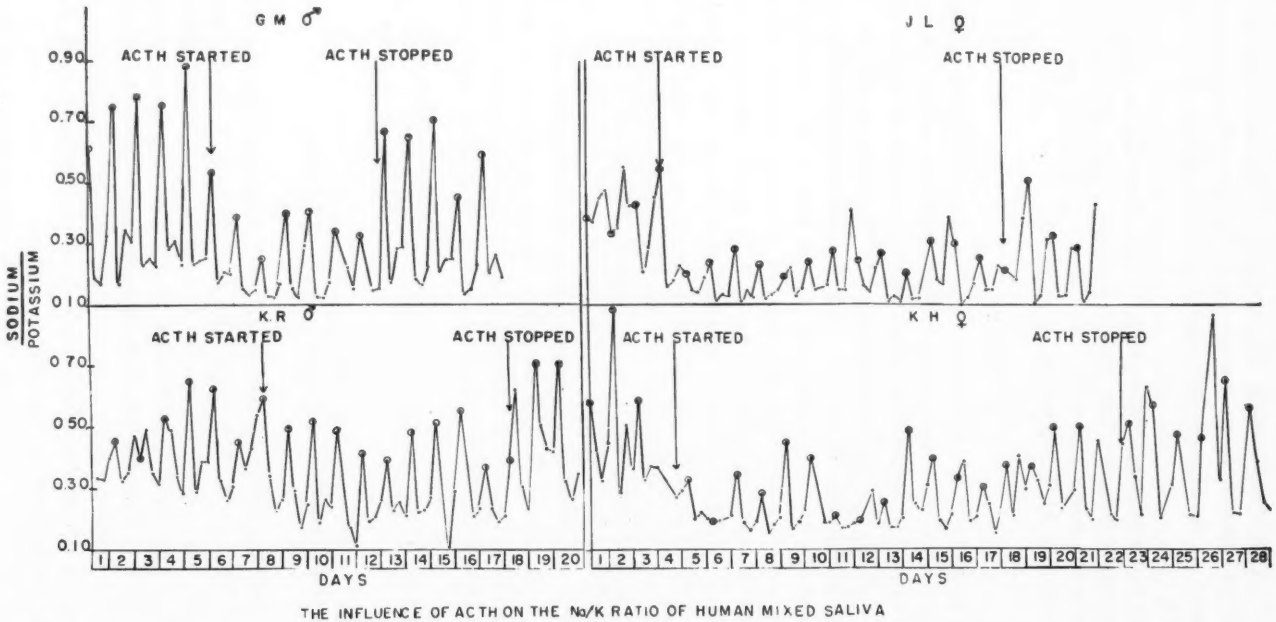

FIG. 5. The saliva specimens collected before breakfast are indicated by the circled dots. Obviously, the great majority of peaks in the Na/K ratio occurred in the "before breakfast" samples. The dosages of ACTH administered are indicated in Figures 1, 2, 3, and 4.

ated via the adrenal cortex because a) the adrenal cortex has a salt-regulating function, the stimulation of which should decrease the Na/K ratio of the saliva (10), b) ACTH did in fact stimulate the adrenal cortex as was apparent in the increase of the urinary 17-ketosteroids and the decrease in circulating eosinophils of K.R. (Fig. 4), and c) the value of the Na/K ratio of the saliva decreased simultaneously (Fig. 4). Hence, a fall or a rise in the Na/K ratio of the saliva can be taken to indicate an increase or a decrease respectively in adrenal cortical salt-regulating activity.

The higher values in the Na/K ratio that occurred before breakfast, compared to the values before the other meals or before retiring (Fig. 5), might be interpreted to indicate a lower level in the salt-regulating activity of the adrenal cortex before breakfast than at the other times of the day or evening. Other workers have demonstrated a similar rhythm in adrenal cortical function, as measured by the urinary excretion of the 17-ketosteroids and the neutral reducing lipids (11). This diurnal rhythm in adrenal cortical function is probably due to a change in the output of ACTH and not to a change in the responsiveness of the adrenal cortex to ACTH, because ACTH was generally as effective in depressing the Na/K ratio of the saliva samples collected before breakfast as at other times of the day. The persistence of the diurnal variation in the salivary Na/K at a lower level throughout the ACTH treatment period suggests that the action of the exogenous ACTH was added to the rhythmical effect of the endogenous ACTH.

The changes brought about by ACTH on the Na and K content of urine, sweat, and saliva were compared by subjecting the data of Forsham *et al.* (2), Conn *et al.* (3) and Locke *et al.* (4) to "*t*" tests in the same way as has already been described for saliva (*see* under "Methods"). Table 1 lists the number of statistically significant responses occurring in the theoretically expected direction, upon the administration of ACTH and upon its withdrawal. The data indicate that the response of the Na and K concentration of saliva was as sensitive to ACTH as that of sweat or as the daily amounts of Na and K of the urine.

Utilizing the data of the present study as well as that of the authors just quoted, the coefficients of variation of the Na and K concentrations of the pre-treatment samples of sweat (3, 4) and saliva, and of the daily amounts of Na and K in the pre-ACTH samples of urine were calculated (2, 3). The day-to-day variation of the Na content of saliva was the same as that of sweat, but greater than that of the urine; whereas that of the K of saliva was significantly less than that of sweat, and the same as that of the K of the urine (Table 1). The relatively slight day-to-day variation in the Na and K concentration of unstimulated saliva collected at the same time of day was all the more surprising, inasmuch as the saliva measurements

were made on patients on an unrestricted Na and K intake, whereas the urine and sweat studies were made on subjects on a fixed daily intake of Na and K (2, 3, 4).

Another advantage in using saliva in these studies was that the collection of the saliva involved only spitting into a bottle during a period of from ten to fifteen minutes, whereas the comparable studies on urine required that 24-hour specimens be collected; and collections of sweat neces-

TABLE 1. COMPARISON OF THE EFFECT OF ACTH ON THE Na AND K OF URINE, SWEAT AND SALIVA

	Urine	*Sweat*	*Saliva*
Reference of study	(3), (4)	(4), (5)	Present study
Nature of study	Rate	Concentration	Concentration
Number of statistically significant changes:			
In mean Na			
Decreases during ACTH therapy	5 out of 7 experiments	4 out of 5 experiments	12 out of 16 experiments
Increases upon withdrawal of ACTH	4 out of 7 experiments	1 out of 5 experiments	5 out of 16 experiments
In mean K			
Increases during ACTH therapy	1 out of 7 experiments	0 out of 1 experiment	8 out of 16 experiments
Decreases upon withdrawal of ACTH	2 out of 7 experiments	0 out of 1 experiment	4 out of 16 experiments
Coefficient of variation (%)			
Na (mean ± standard error)	9±3	33±7	22±2
K (mean ± standard error)	11±3	20±5	8±1

Statistical analyses:

Number of statistically significant changes due to ACTH (χ^2 test):
Na: Saliva *vs.* Urine $0.9 > P > 0.8$; Saliva *vs.* Sweat $P > 0.99$
K: Saliva *vs.* Urine $0.7 > P > 0.5$; Saliva *vs.* Sweat $0.7 > P > 0.5$

Coefficient of variation ("t" test):
Na: Saliva *vs.* Urine $P < 0.001$; Saliva *vs.* Sweat $0.2 > P > 0.1$
K: Saliva *vs.* Urine $0.5 > P > 0.4$; Saliva *vs.* Sweat $0.05 > P > 0.02$

sitated a warming up of at least part of the subject, and special receptacles for collection. The ready availability of saliva allowed more samples to be collected daily, and hence a greater resolution of the effect of ACTH on electrolyte metabolism was made possible (Fig. 5).

These considerations lead to the conclusion that in studies on the effect of ACTH on Na and K, where urine, sweat or saliva can be utilized equally, the saliva is probably the most convenient to use and is equally sensitive.

SUMMARY

Determinations of the Na and K concentrations in specimens of unstimulated saliva collected from 4 patients before meals and at bedtime for several days, before, during, and after ACTH, revealed the following:

a) ACTH significantly reduced the Na/K ratio in 15 out of 16 experiments, this effect being similar in specimens collected at different times of the day.

b) Whether or not ACTH was administered, the Na/K ratio was higher before breakfast than at other times of the day, suggesting a lower output of endogenous ACTH before breakfast than during the day.

c) The response of the Na and K concentration of saliva to ACTH was similar to and as sensitive as that of sweat, or as that of the daily amounts of Na and K in the urine.

d) The day-to-day variation in the Na and especially in the K concentration of unstimulated saliva collected at the same time of day was relatively slight, even though the subjects were on an unrestricted intake of Na and K.

e) The studies presented on saliva are technically simpler than comparable studies on urine or sweat.

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THERAPEUTIC STUDIES IN HYPERTHYROIDISM: METHYLTHIOURACIL

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INTRODUCTION

REPORTS of the experience of others using antithyroid drugs suggest that the administration of these drugs for long periods of time to control hyperthyroidism will often result in permanent remission of that disease. The reported percentage of such remissions varies considerably; some authors report as high as 90 per cent and others as low as 20 per cent (1–5). Frisk emphasizes the increasing number of remissions observed when the treatment is of long duration (3). Because of the rather disappointing experience with propylthiouracil in this clinic (5), it was planned to treat a number of cases of hyperthyroidism with methylthiouracil[1] over a long period of time and determine the incidence of remission after cessation of treatment.

METHODS AND MATERIALS

Seventy patients, of which 60 were female, were studied; the patients were between the ages of 18 and 80, with the majority between 40 and 50 (Table 1). The diagnosis of hyperthyroidism was established by clinical criteria, repeated assays of serum protein-bound iodine, and any other pro-

TABLE 1. AGE AND INCIDENCE OF CARDIOVASCULAR DISEASE AS PRESENTING FEATURE, IN 70 PATIENTS WITH HYPERTHYROIDISM

	Age group						
	Below 20	20–29	30–39	40–49	50–59	60–69	70 plus
Distribution of cases	2	11	17	19	11	8	2
Symptoms or signs of cardiovascular disease, as presenting feature	0	1	1	7	7	4	2

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[1] Methylthiouracil (Muracil ®) was furnished by courtesy of Dr. Kenneth Thompson of Organon, Inc.

cedures such as basal metabolic rate determinations and radioiodine tracer studies, which were needed to confirm the diagnosis. There were 3 pregnant patients in the group. Methylthiouracil was administered in doses ranging from an initial 0.4–0.6 Gm. daily, to doses as low as 0.05 Gm. daily for maintenance.

Arbitrarily, a period of twelve months of therapy was chosen and it was further planned to administer a dosage of the drug sufficient in quantity to render the patients hypothyroid during this period. In addition to the above plan, the drug was necessarily used in the preparation of some patients for other forms of therapy.

The patients were seen at intervals of from two to four weeks when treatment was first started; then, as the disease became less active, at intervals of from four to six weeks. Adequate control was assumed to have resulted when protein-bound iodine assays and the clinical state suggested mild hypothyroidism. The 3 pregnant patients were given smaller doses of methylthiouracil than the other patients, since the aim of therapy in them was to maintain a serum protein-bound iodine level somewhat above normal.

RESULTS

The long-term treatment as outlined was followed fairly satisfactorily by 22 of the 70 patients. Clinical control was achieved in all 22 of the patients and at an average of six weeks after treatment was begun. Of these 22, only 9 maintained a remission after cessation of treatment. These patients, all female, ranged in age from 25 to 80 years; their hyperthyroidism was of varying degrees of severity and there were no clinical features which separated them from the 13 who failed to maintain a remission. The remissions were of six to twenty months' duration. The 13 cases of relapse occurred within six months of stopping treatment.

When therapy with methylthiouracil was stopped the protein-bound iodine level rose in 19 of the 22 cases; in 13 of these, the rise continued and was associated with clinical relapse. Six patients showed a rise in serum iodine levels, followed by a return to normal in 5 (see Table 2). Two patients (Cases 2 and 6) became clinically euthyroid and had normal basal metabolic rates. The rise in protein-bound iodine was above normal in 5 of the 6 cases. Intervals of from three to twenty-two months elapsed before the iodine returned to normal in these instances.

While all patients were well controlled clinically, an effort was made to study the effectiveness of control in terms of protein-bound iodine levels. Table 3 shows the length of time during treatment that the protein-bound iodine levels were kept below 5 micrograms per cent, in both the relapse and the remission cases. The difference raises the question whether the patients who maintained a remission were treated more vigorously. The

TABLE 2. THE COURSE OF SERUM PROTEIN-BOUND IODINE LEVELS IN 9 PATIENTS WHO MAINTAINED A REMISSION AFTER THERAPY WAS STOPPED

Patient	PBI at time methylthiouracil was stopped (micrograms %)	PBI in succeeding months (micrograms %)
1	5.5	9.5 (2 months later)..... 6.2 (6 months later)
2	4.6	13.8 (5 months later)..... Gradual fall to 4.8 (22 months later)
3	3.7	6.7 (7 weeks later)..... 7.7 (13 weeks later)
4	3.7	8.4 (4 weeks later)..... 6.1 (6 months later)
5	1.6	8.6 (7 weeks later)..... 6.2 (11 months later)
6	5.0	10.2 (12 months later).... Clinically euthyroid
7	6.1	6.0 (2 months later)..... No rise in following months
8	2.0	2.1 (1 week later)..... 2.4 (3 months later)
9	0.5	2.8 (2 weeks later)..... 5.0 (6 months later)

TABLE 3. THE LENGTH OF TIME DURING TREATMENT THAT THE SERUM PROTEIN-BOUND IODINE WAS KEPT BELOW 5 MICROGRAMS PER CENT, IN THE PATIENTS WHO SUBSEQUENTLY MAINTAINED A REMISSION OR HAD A RELAPSE OF THE DISEASE

Remission (9 cases)	Relapse (13 cases)
Average ..... 6 months	Average..... 3.6 months
Range.....(2–11 months)	Range.....(0–12 months)

TABLE 4. THE AMOUNT AND DURATION OF TREATMENT WITH METHYLTHIOURACIL IN A GROUP OF PATIENTS WHO MAINTAINED REMISSION AND IN A GROUP WHO HAD A RELAPSE OF THE DISEASE

	Average total dose and range, in grams	Duration of treatment in weeks (average and range)
Remission (9 patients)	85.9 (39–109)	51.9 (34–65)
Relapse (13 patients)	97.7 (44.5–151.3)	61.5 (41–79)

figures in Table 4 show that the patients who suffered a relapse had a total dosage of as much or more of the drug and for a longer time on the average than did those who maintained a remission. This would suggest that the patients who have a relapse are perhaps harder to control, and in some as yet undefined way resist the effect of the antithyroid drugs.

In 7 of the 70 cases the drug was stopped, in view of untoward side-reactions.

In 2 of the 70 cases, methylthiouracil was temporarily used as a substitute for propylthiouracil.

In 7 of the 70 cases, the patients failed to continue the treatment.

In the remaining 32 of the 70 cases, the drug was administered for varying periods of time, at the conclusion of which some other form of therapy was carried out. In 16 of this group, other therapy was instituted partly in consideration of the patients' dissatisfaction with the long-term plan as outlined.

Toxic reactions

In 7 of the 70 cases of hyperthyroidism in this study conditions developed which were severe enough to result in cessation of methylthiouracil therapy. They are outlined briefly as follows, under the headings of the system affected:

I. *Gastro-intestinal*

Case #23. Female, aged 30. Initially, in addition to hyperthyroidism, evidence of liver disease was noted. She received propylthiouracil (300 mg. daily) for one month, following which she received methylthiouracil, 400 mg. daily. When two months had elapsed, nausea, vomiting, jaundice, etc. occurred and a diagnosis of acute hepatitis was made.

Case #24. Female, aged 40. Methylthiouracil (400 mg. daily) was given for two months, followed by onset of nausea, vomiting, and fever, with a white blood count of 5,900. No definite diagnosis.

Case #25. Female, aged 39. After three months of receiving 300–400 mg. of methylthiouracil daily, jaundice occurred, and a diagnosis of acute hepatitis was made. There was no evidence to suggest pre-existing liver disease.

Case #26. Female, aged 33. Several weeks after starting 400 mg. of methylthiouracil daily, she became pregnant. The hyperthyroidism was controlled ultimately with only 50 mg. daily; but while taking this dosage premature labor occurred at 7 months. Using ether and later chloroform anesthesia, a viable infant was delivered. On the following day, however, jaundice was noted and a diagnosis of hepatitis was subsequently made. There was no antecedent history of findings suggestive of liver disease.

II. *Integumentary system*

Case #27. Female, aged 46. After six months of ingesting decreasing quantities of methylthiouracil, during which period the hyperthyroidism was well controlled, it was noted that the patient's hair was becoming sparse. Treatment was continued for a further three to four months, during which period the concentration of serum protein-bound iodine ranged between 1.2 and 3.7 gamma per cent. The patient ultimately was receiving 25 mg. daily. Because of the loss of hair, treatment was stopped; vaginal bleeding had been noted shortly before the drug was omitted, but other than erosion of the cervix, no local disease was found.

III. *Locomotory system*

Case #28. Female, aged 47. From two to three weeks after commencing methyl-

thiouracil (400 mg. daily) she complained of swelling and pain of the small joints of hands but nothing of note was found on examination. Still later, the patient had difficulty opening the hands, and a condition resembling Dupuytren's contractures was noted; this was followed by pain about the shoulders. Still later, with the appearance of what the patient described as a "numb feeling in the face" and numerous other unaccountable symptoms, the drug was stopped. The symptoms, except for the arthralgia, continued after omission of therapy.

IV. *Hematopoietic system*

Case #20. Female, aged 67. After a thyroidectomy in 1947, the patient noticed increasing prominence of the eyes. She was found to have a marked degree of exophthalmos and some residual hyperthyroidism in 1948 and treatment consisted of propylthiouracil, stilbestrol and thyroid substance. Later, corneal ulcers were treated and still later methylthiouracil was substituted for propylthiouracil. After receiving decreasing doses of methylthiouracil for about fifteen months, the patient complained of increasing weakness, and later a marked anemia of a hypochromic microcytic type was noted. A thorough investigation did not reveal the cause of the anemia, and at no time were the white blood cells affected. Methylthiouracil was, however, stopped and radioiodine was given. The anemia has spontaneously improved.

DISCUSSION

Seventy cases of hyperthyroidism are presented, and diagnostic criteria and accompanying conditions are noted. It is interesting to note (Table 1) the incidence of heart disease as the presenting feature in a large number of the cases, rather than the classic symptoms of hyperthyroidism.

It is quite possible that of the toxic reactions encountered, most were coincidental and not related to methylthiouracil. However, the instances of jaundice associated with hepatitis (3 cases) appear significant. The observations of Barfred (4) on the incidence of increased urobilinuria as a toxic manifestation of the drug may be related. A case of hepatitis associated with thiouracil therapy has been reported by Holobeuk, *et al.* (6). Moore, reporting on over 1,000 cases of thiouracil administration similarly reports this complication (7). Leukopenia and agranulocytosis were not encountered in the cases studied; but this complication has been reported by others (7) and a case of fatal agranulocytosis due to methylthiouracil has been reported by Treanor *et al.* (8).

In 60 per cent (13 of 22) of the patients in whom treatment was sustained for approximately twelve months, relapse occurred up to six months later. If one considers that of the original 70 cases, 52 were originally started on the long-term plan of treatment; and that of these, only 22 completed the course, then the incidence of success (9 out of 52) becomes even lower. These poor results are at variance with those of some other workers. The lack of success may be based on too short a period of administration of the antithyroid drug. However, the major defect lies in the number of patients in this group who failed to complete the treatment. The intense hy-

perplasia of the thyroid induced by thiouracil derivatives conceivably could act as a perpetuating mechanism for hyperthyroidism; therefore, if a high percentage of remissions of hyperthyroidism is to be achieved with methylthiouracil, the drug should be continued until some sort of "exhaustion atrophy" of the thyroid takes place. The means of determining such a histologic change is not easy; serial biopsy specimens from the thyroid might be helpful.

CONCLUSIONS

1. Methylthiouracil has been administered to a group of 70 patients with hyperthyroidism.
2. Control of the disease was evident in about six weeks.
3. Seven patients had coincident reactions, because of which the methylthiouracil was stopped. A causal relationship between the antithyroid drug and the reactions was not clear.
4. Thirteen of 22 patients who received methylthiouracil for twelve months or longer had a relapse of hyperthyroidism when the drug was omitted.

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INTERSTITIAL CELL TUMOR OF THE TESTIS. STUDY OF A 5-YEAR-OLD BOY WITH PSEUDO-PRECOCIOUS PUBERTY*

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THE interstitial cells of the testis, which were first described by Leydig (1), occasionally may give rise to tumors. Eight such cases occurring in childhood have been described in the literature (2–9). In the pediatric age group these tumors occur mostly during the fifth and sixth years of life and apparently are invariably associated with signs of precocious masculinization. The present report describes an additional case of pseudo-precocious puberty due to a testicular interstitial cell tumor in a 5-year-old boy, and gives quantitative and qualitative information concerning his urinary steroid excretion.

REPORT OF CASE

This white boy, aged 5 3/12 years, was admitted for study because of precocious masculinization. Eight months earlier, it had been noted that his voice had become deeper, that he had suddenly begun to gain rapidly in height and weight, and that his penis was becoming larger than normal. At the same time, acne was seen on his face. Seven months before admission, pubic and axillary hair were first noticed, and hair appeared on his upper lip. One month prior to admission, his mother noted a mass in the right scrotum. This swelling increased somewhat in size during the ensuing month. There had been no known erections or emissions and no pain or tenderness in the scrotum. No urinary symptoms were elicited. The child, who previously had behaved normally, became progressively more hyperactive and aggressive. The family history and the patient's past history were noncontributory. The patient's growth rate and development had been considered normal by the family until the onset of the present illness, and he appeared to be of average intelligence.

Physical examination showed a large, muscular boy 133 cm. tall, weighing 35.5 Kg. (Fig. 1). Height and weight were consistent with those of a normal boy 9 3/12 years. There was a prominent acneiform eruption over the face, forehead, and shoulders. The findings on funduscopic examination were within normal limits, and confrontation

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visual fields were normal. The heart and lungs were normal, but the blood pressure was slightly elevated, averaging 138/90 mm. of mercury on repeated determinations. There was no gynecomastia. The abdomen was normal. There was abundant axillary hair and a triangular distribution of pubic hair, which was black and coarse. On the upper lip there was a moderately heavy growth of fine hair. The penis was 8 cm. in length, and the scrotum was like that of an adult, with increased vascularity, pigmentation,

FIG. 1. Preoperative photograph showing right testicular swelling, virilism, and development of secondary sexual characteristics.

and wrinkling of the skin. The left testis measured 1 by 2 cm. and was of normal consistency; the epididymis was normal on palpation. The right testis was enlarged to 4 by 6 cm., and was smooth and firm on palpation. The epididymis on the right could be palpated, and apparently was normal. The prostate was palpably enlarged, but normal in consistency.

Roentgen examination of the skull, abdomen, and each hand and wrist showed normal findings except for marked advancement of the bone age, which corresponded to a standard of approximately 13½ years. A chest film showed no metastases or changes from normal; an intravenous pyelogram showed nothing abnormal.

Pertinent laboratory data are included in Table 1. Preoperatively, there was a moderate elevation in serum sodium and chloride values, and a tendency toward eosinopenia. The most striking laboratory finding was the markedly elevated urinary 17-ketosteroid output (16.8 mg. in twenty-four hours), the upper limit of normal being approximately 1 mg. for children of this age (10). The urinary 11-17-oxycorticosteroid output was normal, 0.097 mg. in twenty-four hours.

Figure 2 shows the values obtained after chromatographic separation of an 0.3-mg. aliquot of the total 17-ketosteroids into 41 fractions, by the method of Zygmuntowicz and her associates (11). The results obtained for this patient are indicated by the continuous line. These may be compared with average values for normal adult males, indicated by the broken line. The shape of the curves is determined by the values for the per cent of total 17-ketosteroids appearing in each fraction. The height of the normal

TABLE 1. PERTINENT LABORATORY DATA FROM CASE REPORTED HERE

	Before surgery	After surgery	1 Month later	13 Months later
Blood pressure, mm. of mercury	138/90	120/78	128/88	108/84
Serum sodium, mEq. per liter	154	151	146	
Serum chloride, mEq. per liter	112	98	107	
Nonprotein nitrogen, mg. per 100 cc.	35.4			
Blood sugar, mg. per 100 cc.	102			
Eosinophils per cubic millimeter	0–27	88	99	
Urinary 17-ketosteroids, mg. excreted in 24 hrs.	16.8	1.13	0.273	0.99

curve was determined by assuming an average total urinary 17-ketosteroid output for normal adult men equal to that of the patient (16.5 mg. per day). This assumed value is somewhat above the actual adult male value of 11 mg. per day reported previously (10). It is seen that the patient excreted much more 17-ketosteroid material of the types which appear in area III and appreciably less of the types which are eluted in areas I, II, IV, and VI, than are excreted by normal men. The patient had a small peak in Fraction 4, which lies at the boundary of areas I and II. Digitonin precipitation of an aliquot of the patient's urinary 17-ketosteroids failed to yield any 3-β-OH-17-ketosteroids. Chromatographic fractionation of the 3-α-OH-17-ketosteroids remaining after digitonin precipitation yielded a curve essentially identical to that shown in Figure 2. Hence, it appears reasonable to assume that the peaks shown in this curve are attributable exclusively to 3-α-OH-17-ketosteroids.

After completion of these studies, removal of the right scrotal mass was carried out. The spermatic cord was ligated at the internal ring, and the cord and tumor mass were removed as a unit, without entering the tumor at any time. Preparations were made to provide substitution endocrine therapy had the tumor suppressed normal adrenal function to the point where temporary hypoadrenocorticism resulted from its removal. However, the boy tolerated the procedure well, the postoperative period was uneventful, and he was discharged home on the eighth postoperative day. As can be seen from Table 1, the 17-ketosteroid output had fallen, and the eosinophil count had risen to normal levels by the time of discharge from the hospital.

Thirteen months postoperatively, the child's general appearance had not changed

significantly. However, the muscularity was slightly less marked, and the boy was said to be less aggressive than he had been. The deep voice was still present. The acne had improved only slightly, and the axillary and pubic hair were unchanged, as was the size of the penis. The blood pressure was within normal limits (108/84 mm. of mercury). The 17-ketosteroid output had remained normal.

Pathologic description. The specimen consisted of a spermatic cord and a firm, swollen

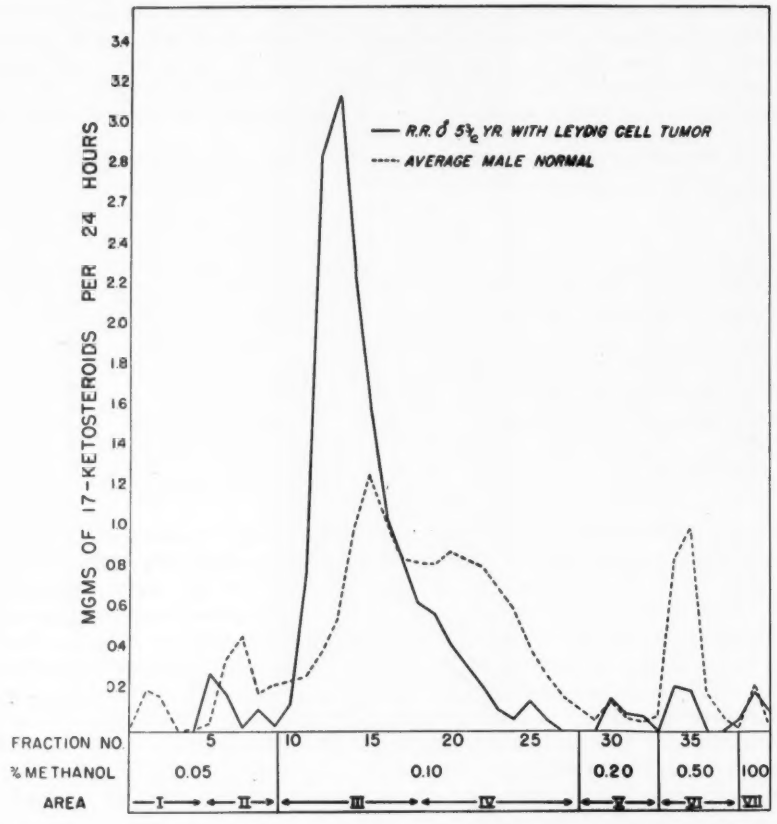

FIG. 2. Chromatographic pattern of patient's urinary 17-ketosteroids preoperatively (solid line) compared to the pattern for average adult male (dotted line).

testis measuring 4.5 by 3.5 by 2.8 cm. and weighing 46 Gm. The tunica albuginea was normally smooth, white, and glistening. On section, the tissue bulged moderately. The cut surface was uniformly finely lobulated and gray-brown, with scattered yellow foci measuring 0.1 to 0.5 cm. in diameter. No invasion of the tunica was noted, nor was there extension or metastasis of the tumor to the spermatic cord.

Microscopically (Figs. 3 and 4), the specimen consisted almost entirely of closely packed, large rounded to polygonal cells with faintly acidophilic, finely granular cyto-

plasm, containing occasional small vacuoles. Careful search failed to reveal any crystalloids of Reinke. Usually the nuclei were small in relation to the amount of cytoplasm, with poorly defined nuclear membranes and chromatin pattern, and with single small central nucleoli. A moderate number of binucleate cells and rare multinucleate cells were present. In addition, a few cells had enlarged, round or irregular nuclei, many of

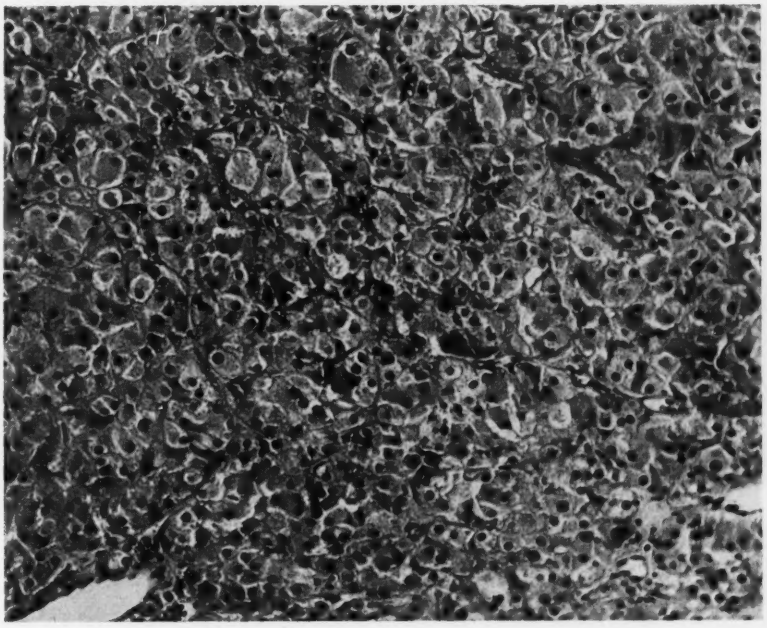

FIG. 3. Photomicrograph of tumor showing its general architecture. The closely packed cells are large and have small nuclei. (Hematoxylin and eosin, ×175.)

which were hyperchromatic, and a few of which contained large, round, acidophilic intranuclear inclusions. No mitoses were seen. The tumor cells were divided into poorly defined lobules by a scanty collagen stroma, and many microscopic foci of perivascular calcification were seen. No testicular tubules were present within the tumor, which was surrounded by a thin, intact capsule of fibrous tissue.

Unstained frozen sections showed a faint brown color in the cytoplasm of the tumor cells; no doubly refractile material was present. Sudan IV stain for fat showed multiple small fat droplets in a few cells and single large vacuoles in rare ones, but the majority contained no stainable material. With phosphotungstic acid-hematoxylin stain, the cytoplasm of the tumor cells contained large numbers of deep purple granules, most numerous in the periphery of the cell, and in sections stained by Mallory's aniline blue procedure, the cytoplasm was uniformly strongly fuchsinophilic. Ziehl-Neelsen stain showed the cells to contain no acid-fast material. Best's carmine stain for glycogen and the periodate-leucofuchsin stain for carbohydrates showed the cells to contain no material reacting with the former, and only faint central coloration with the latter. The Ashbel-Seligman reaction for carbonyl groups was negative in the majority of the

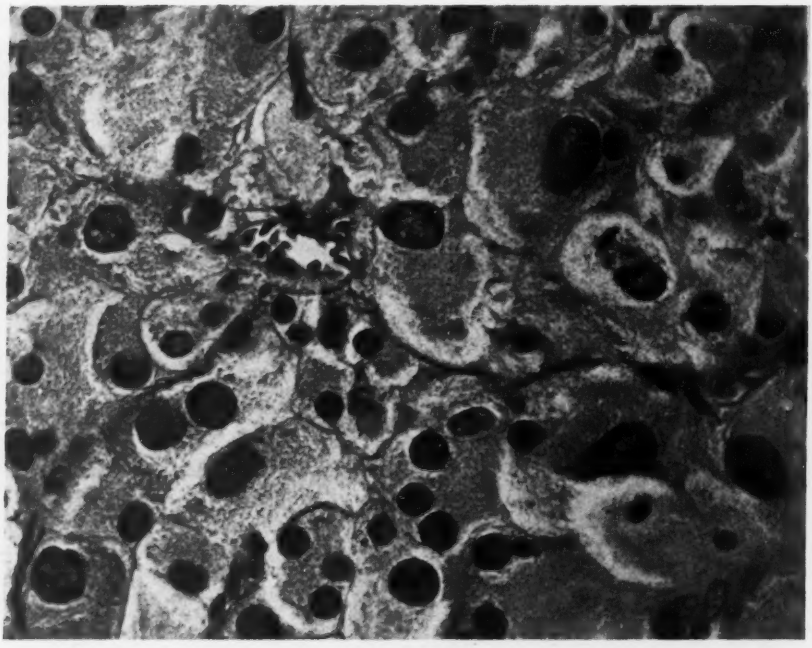

FIG. 4. Photomicrograph of tumor showing the variation in size and shape of both cells and nuclei and the finely granular cytoplasm with central darker staining zone. The close resemblance to interstitial cells is well shown. (Hematoxylin and eosin, ×600.)

TABLE 2. CASES OF TESTICULAR INTERSTITIAL CELL TUMOR REPORTED IN CHILDREN

	Age at onset	Age at operation	Pseudo-precocious puberty	Postoperative course
Sacchi (2)	5 6/12	9 6/12	Present	Voice reverted; considerable regression
Rowlands *et al.* (3)	6	9	Present	No regression
Stewart *et al.* (4)	4	5	Present	Pubic hair disappeared
Somerford (5)	5	11	Present	No regression
Huffman (6)	4	6	Present	No regression
Urban (7)	5	5 1/12	Present	Pubic hair disappeared
Werner *et al.* (8)	5 6/12	6 9/12	Present	Moderate regression
Sandblom (9)	2 3/12	3 6/12	Present	Regression
Cook *et al.*	4 7/12	5 3/12	Present	Slight regression

cells, but occasional ones did contain small blue droplets. The cytoplasm of the tumor cells gave a strongly positive Gömöri alkaline phosphatase reaction.

Several sections showed a small area of testicular tissue separated from the tumor mass by a fibrous capsule. The tubules were uniformly larger than normal for the age of the patient, with well-formed lumens; the interstitial tissue was loose and moderately abundant. No Leydig cells were seen between the tubules. Many tubules showed active division of primary spermatocytes (Fig. 5), and a smaller number showed division of

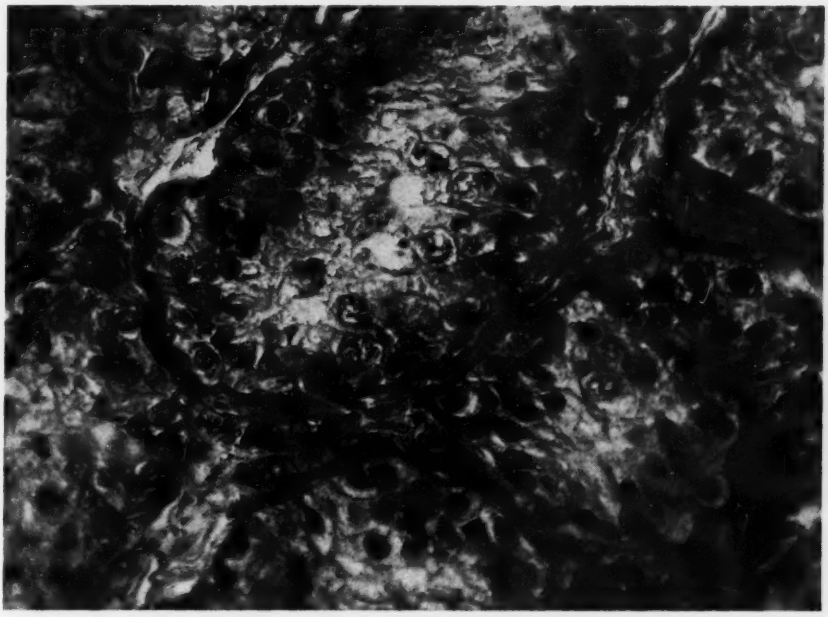

FIG. 5. Testicular tubules showing well formed lumens, scant interstitial tissue, and prophase figures in primary spermatocyte layers. There is some stimulation of the secondary spermatocytes, but no maturation to spermatids or spermatozoa. (Phosphotungstic acid-hematoxylin, ×700.)

spermatogonia and of secondary spermatocytes. No evidences of maturation to spermatids or spermatozoa were seen, and the tubules of the epididymis contained only small amounts of precipitated secretion. The tubules of the paradidymis were also unusually large, and lined by high columnar epithelium.

DISCUSSION

A ninth case of interstitial cell tumor in a child is reported. As in all previous cases (Table 2), the tumor was unilateral, nonfamilial, and associated with pseudo-precocious puberty, namely, development of adult male sex characteristics without functional sexual maturity (formation of mature

sperm). An interesting feature of this rare condition is the apparent restriction to a narrow age range. All reported pediatric cases occurred in children between 2 3/12 and 6 years of age, with 7 of the 9 occurring in the fifth or sixth years of life.

This tumor was typical of the previously reported benign interstitial cell tumors of childhood in its unilaterality, color and gross appearance, microscopic appearance and staining properties of the cells, and the absence of crystalloids of Reinke. The faint staining with Sudan IV and the Ashbel-Seligman reaction, and the absence of doubly refractile material, indicate that the tumor cells were not storing significant amounts of steroids, but presumably were releasing them as rapidly as they were synthesized. The high alkaline phosphatase content of the tumor is of interest, in view of the presence of multiple foci of calcification; such foci have been reported previously in a tumor of this type (6). The fuchsinophilia of the cells and their purple color with the phosphotungstic acid-hematoxylin stain are both typical features of Leydig cells.

Typical clinical signs of androgenic hormone action which this patient showed were enlargement of the penis, changes in the scrotum, enlargement of the prostate, increased muscularity, increased skeletal growth and skeletal maturation, adult type axillary and pubic hair, and acne. Another possible effect of androgenic hormone is the testicular tubular development shown in this patient, and also in Stewart's patient (4). In that relation, both in experimental animals (12) and in man (13), testosterone has been shown to stimulate the testicular tubules directly; and currently available evidence suggests that Leydig cells normally produce, among other androgens, testosterone (14, 15).

Testosterone is known to be metabolized principally to androsterone and etiocholanone (16), substances which appear in areas III and IV, so that the chromatographic findings on the urine of this patient help to substantiate the hypothesis that this tumor produced testosterone. Furthermore, Hoffman (17), in studying the urine of an adult patient with a malignant interstitial cell tumor of the testis, found that particularly excessive amounts of androsterone and etiocholanone were excreted.

The hypertension, the elevation in serum sodium and chloride levels, and the eosinopenia are changes not usually associated with androgenic activity. However, Thorn and Harrop (18) and Kenyon and his associates (19) reported sodium retention when testosterone was administered to normal adults and eunuchoid individuals. These observations are interpreted to suggest that the tumor in this patient was producing a hormone or hormones resembling testosterone in androgenic action, but possibly differing from it in some other metabolic effects. For example, testosterone apparently is incapable of inducing eosinopenia (20). Thus, the occurrence

of eosinopenia in this patient suggests that the tumor may have been producing at least small amounts of adrenocorticosteroids, but in this connection it is noteworthy that the urinary output of 11-17-oxycorticosteroid-like substances was not abnormally increased. Moreover, in this patient and in those described by others, no signs of hypoadrenocorticism developed postoperatively.

A unilateral tumor, such as occurred in this patient, apparently arises spontaneously and independently of pituitary stimulation. The fact that the Leydig cells in the remaining portion of the testis were not hyperplastic is added, but certainly not conclusive, evidence that there was no central stimulation. In the cases of unilateral interstitial cell tumors in children reported in the literature, operative removal has always stopped the progression of the virilizing process, and in 6 of the 9 cases, slight to considerable regression of the precocity occurred. In our case, removal of the tumor resulted in a prompt and sustained drop to normal in the urinary 17-ketosteroid output.

The chromatographic pattern of the urine in Leydig cell tumors may be helpful in diagnosis, if more cases can be shown to produce similar results, because most of the excessive urinary output of 17-ketosteroids fell into the fraction identified with androsterone, namely, area III of Figure 2. In contradistinction, the chromatographic pattern of the urine of patients with adrenocortical virilism shows a more general increase in 17-ketosteroid output, particularly in fractions 10 and 35 and area VII, in addition to an increase in area III.

SUMMARY

A ninth case of interstitial cell tumor associated with pseudo-precocious puberty in a child is reported, along with a pathologic description of the tumor and postoperative follow-up data. Chromatographic studies of the excessive urinary output of 17-ketosteroids showed that these were predominantly 3-α-OH-17-ketosteroids. Operative removal of this benign tumor resulted in cessation of the abnormal androgenic activity. This type of tumor in children appears to be limited to the period between 2 and 6 years of age, and the clinical picture seems to be uniform.

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A NEW AND SIMPLIFIED PROCEDURE FOR THE DETERMINATION OF FREE PREGNANEDIOL IN URINE, AND ITS EVALUATION*

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THE estimation of urinary pregnanediol serves as a useful index of progesterone metabolism in the problems of pregnancy and menstruation. Since the isolation of pregnanediol by Marrian (1) and its later identification by Butenandt (2), two basic methods for its measurement have been devised. The first of these utilizes the pregnanediol glucuronidate complex and is expressed in terms of pregnanediol (3). The second determines free pregnanediol directly following the hydrolysis of the pregnanediol complex (4). Continuing studies have suggested that discrepancies exist between the quantity of pregnanediol excreted when measured by the two methods. It seems fairly well substantiated at the present time that in pregnancy the determination of the free pregnanediol yields significantly lower values (5). It would appear from present-day literature that methods utilizing the measurement of free pregnanediol have become the more popular of the two. In spite of many minor modifications, the results obtained with this method have been more or less uniform.

Since pregnanediol is a relatively inert steroid, showing few and nonspecific color reactions, its determination in small quantities in the presence of larger quantities of other steroids is attended with some difficulties. Primary reliance must be placed on its isolation in as pure a state as possible, so that the quantitative determination does not include other steroids or chromogens.

The procedure described is designed to give particularly good separations consistent with reasonable time requirements for a clinically useful technique.

The principal innovations are the use of iso-octane as an extraction solvent, the substitution of concentrated aqueous sodium hydroxide for methanolic sodium hydroxide in purification, followed by chromatographic adsorption and elution, and the use of spectrophotometric measurement at two or more wavelengths as a criterion of purity.

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The clinical results seem to justify the use of these steps, since much lower results are obtained in urine specimens containing minimal quantities of pregnanediol than given by other currently used procedures. At the same time, recovery of added pregnanediol is at a very high and satisfactory level, indicating that losses have been held to a minimum.

The detailed procedure for the determination as devised by Albert L. Chaney and William E. McKee is presented here.

PROCEDURE

Reagents: The required solvents, redistilled in glass, are iso-octane (pure grade, Phillips Petroleum Co. or Enjay Company, Inc.), toluene (reagent grade), and methanol (reagent grade).

Reagent grade sulfuric and hydrochloric acid are used.

Sodium hydroxide solutions are prepared, 50 per cent and 10 per cent by weight, respectively. The former is filtered free from carbonate.

The adsorption medium consists of a mixture of three parts by weight of Celite 545 (Johns-Manville) and one part calcium carbonate (Merck's Precipitated, U.S.P.). The Celite is previously heated at 150° C. for four hours. The two materials are intimately mixed in a flour sifter and then shaken for a few minutes in a widemouth bottle containing some loose rubber stoppers for agitation.

Extraction and purification: Fifty ml. of urine (or a smaller aliquot) and 25 ml. of iso-octane are heated to boiling in a 500-ml. Kjeldahl flask with a cold finger condenser, and 5.5 ml. of concentrated hydrochloric acid are then added. After ten minutes' hydrolysis and reflux, and cooling to 60° C., the contents are transferred to a separatory funnel. The aqueous layer is returned to the extraction flask for a second ten-minute period of refluxing with an additional 25 ml. of iso-octane, which is combined with the first extract. The aqueous layer is given a third extraction by shaking vigorously with 25 ml. of iso-octane, which is added to the previous extracts.

During the extraction and washing procedure, the contents of the separatory funnel are kept warm by an infra-red lamp (50°–60° C.) and all wash solutions are first heated to 50°–60° C. before use. Washing of the combined iso-octane extract to remove acidic materials is carried out three times with 25-ml. portions of 10 per cent NaOH, once with distilled water to remove carbonate, and then with a 10-ml. portion of 50 per cent NaOH (shaking vigorously for two minutes). All scum should be removed at this time, before traces of alkali are removed by three 25-ml. water washes.

Twenty ml. of toluene are added to the washed iso-octane extract and the mixture is evaporated in the hood to a volume of about 25 ml. to remove dissolved and entrained water; it is then cooled to room temperature.

Chromatography is carried out in a Tswett column, 2 cm. in diameter and 20 cm. high, containing 11.0 grams of Celite-calcium carbonate mixture gently packed under suction. The extract in iso-octane and toluene is poured onto the column, using 10 inches of mercury vacuum, and washed with three 20-ml. portions of toluene and one 10-ml. portion of toluene containing 0.2 per cent methanol (vol./vol.). These washings are discarded.

The pregnanediol is eluted from the column with 50 ml. of toluene containing 0.2 per cent methanol. This fraction is evaporated on a water bath or hot plate nearly to dryness (3–5 ml.). Scorching is carefully avoided. Evaporation is completed with a gentle filtered air stream on a water bath. The cooled and dry residue is washed with

two 1-ml. portions of iso-octane in the cold, to remove some ketosteroids not separated by chromatography. The iso-octane washes are removed by a capillary pipette, and the residual solvent is evaporated to dryness on a water bath. After cooling to room temperature, colorimetric determination is made by dissolving the pregnanediol in 10 ml. of concentrated sulfuric acid and developing the color for thirty minutes at 25° C.

Measurements of color intensity are made in a Beckman spectrophotometer (Model B or DU) at 425 mμ, and also at 390 and 460 mμ. Standards are prepared containing .02–.75 mg. of pure pregnanediol in 10 ml. of sulfuric acid. For reasonable purity, the ratio of optical densities for the specimen should be as follows:

$$\frac{\text{Density at 425 m}\mu}{\text{Density at 390 m}\mu} = 1.5 \text{ approx.} \qquad \frac{\text{Density at 425 m}\mu}{\text{Density at 460 m}\mu} = 1.8 \text{ approx.}$$

RECOVERY DATA

Crystalline pregnanediol: To test this method, recovery experiments were performed. Pooled male urine served for the controls as well as for the test media for addition of either pregnanediol or sodium pregnanediol glucuronidate.[1] For all determinations, 50-ml. aliquots of urine were used. The highest concentration of pregnanediol added was 0.773 mg., and the highest concentration of sodium pregnanediol glucuronidate added was 1.50 mg. Crystalline pregnanediol in 95 per cent ethanol was added at two different points of the procedure: 1) before acid hydrolysis of the urine, and 2) to the combined iso-octane extracts after hydrolysis. By comparison of the recovery data of Table 1 (parts A and B of each experiment, and the column headed "Recovery of part A minus part B") it can be seen that there is no appreciable loss of added crystalline pregnanediol during the combined acid-hydrolysis and extraction step. The maximum loss in recovery shown between adding pregnanediol before or after acid hydrolysis and extraction of the urine, is 0.05 mg. (Table 1 Experiment 4), or approximately 7 per cent of the theoretical recovery. The authors believe that this amount should not be considered as destroyed by acid hydrolysis, but as lost due to technical manipulation throughout the entire procedure. To substantiate this belief, Experiments 1 and 2 in Table 1 show higher recoveries of pregnanediol when added before acid hydrolysis than when added after acid hydrolysis of the urine. The loss of pregnanediol with this procedure appears to occur during the purification procedure.

Sodium pregnanediol glucuronidate: As the purity of the sodium pregnanediol glucuronidate supplied us was unknown, we were uncertain as to what factor should be used to compute recoveries as pregnanediol. The glucuronidate gave an intense positive Zimmermann reaction indicating a ketone, probably pregnane-3(α)-ol-20-one (6, 7) present as an impurity.

[1] Crystalline pregnanediol and crystalline sodium pregnanediol glucuronidate were courteously supplied by Ayerst, McKenna and Harrison, Ltd., for this investigation.

Based upon the observation of Semmons and McHenry (8) that pregnanediol is the only part of the sodium pregnanediol glucuronidate molecule that gives color with sulfuric acid, and the observation of Marrian and Gough (6) that pregnane-3(α)-ol-20-one is only feebly chromogenic in sulfuric acid, the following experiment was performed to determine the purity

TABLE 1. RECOVERY OF ADDED PREGNANEDIOL

Pooled male urine sample (50 ml.)	Pregnanediol added before hydrolysis (mg.)	Pregnanediol added after hydrolysis (mg.)	Actual recovery as pregnanediol (total recovery minus urine blank) (mg.)	Pregnanediol lost (mg.)	Recovery of part A minus part B (mg.)	Overall recovery of complete procedure (%)
Expt. 1						
Part A	0.000	0.126	0.070	0.056		55.6
Part B	0.126	0.000	0.071	0.055	—	56.3
Expt. 2						
Part A	0.000	0.286	0.220	0.066		76.9
Part B	0.286	0.000	0.240	0.046	—	83.9
Expt. 3						
Part A	0.000	0.523	0.501	0.022		95.8
Part B	0.523	0.000	0.472	0.051	0.029	90.2
Expt. 4						
Part A	0.000	0.773	0.669	0.104		86.5
Part B	0.773	0.000	0.619	0.154	0.05	80.1

Average blank for Expts. 1–3: 0.022 mg. as pregnanediol
Average blank for Expt. 4: 0.029 mg. as pregnanediol

of the sodium pregnanediol glucuronidate used by us, and from this purity to compute a conversion factor for recoveries. Sodium pregnanediol glucuronidate as supplied was dissolved in 90 per cent ethanol (1.0 mg./5.0 ml.). Aliquots representing 0.1 mg. to 1.5 mg. of sodium pregnanediol glucuronidate were transferred to spectrophotometer cuvettes and cautiously evaporated to dryness in a warm water bath with vacuum. To the dry residue in each tube was added 10 ml. of concentrated sulfuric acid; the material was mixed with a glass stirring rod and then allowed to stand for thirty minutes, at which time the tubes were read at 425 millimicrons, and the readings recorded as milligrams of pregnanediol from a reference preg-

nanediol curve.[2] Table 2 gives the actual values found, assuming that 1.0 mg. of sodium pregnanediol glucuronidate.$3H_2O$ (5), if 100 per cent pure, would give a value of 0.559 mg. as pregnanediol. The average purity of our sample was 75.2 per cent, giving a factor of 0.420 for the conversion of sodium pregnanediol glucuronidate to pregnanediol. This factor applies to one sample of sodium pregnanediol glucuronidate only and it is not intended as a generally acceptable conversion factor for all sodium pregnanediol glucuronidate samples. As the purity of this sample is in the range of 80 per cent, which is the purity generally reported for sodium pregnanediol glucuronidate (6, 7), the authors feel that our factor is more accurate for this sample of sodium pregnanediol glucuronidate than an assumption of 80 per cent purity.

TABLE 2. PURITY OF SODIUM PREGNANEDIOL GLUCURONIDATE

Cuvette number	Sodium pregnanediol glucuronidate (mg.)	Found as pregnanediol (mg.)	Theoretical recovery, if 100% pure (mg.)	Purity, %
1	0.10	0.056	0.056	100.0
2	0.20	0.073	0.112	65.2
3	0.25	0.102	0.140	72.9
4	0.50	0.221	0.280	78.9
5	1.00	0.398	0.559	71.2
6	1.50	0.530	0.839	63.2
				Av. purity 75.2

Factor: $0.752 \times 0.559 = 0.420$.

Table 3 shows the recovery values when sodium pregnanediol glucuronidate in 90 per cent ethanol was added to urine before acid hydrolysis. Recovery of sodium pregnanediol glucuronidate as pregnanediol averages 88 per cent.

DISCUSSION

The present analytic method was worked out using the pregnanediol 3-alpha, 20-alpha isomer, but as pregnanediol has not been detected in any fractions of the chromatogram of urine extract other than the one in which the 3-alpha, 20-alpha occurs, it has been assumed that the total of the stereoisomers is being determined.

[2] Coleman Model 14 spectrophotometer with 19-mm. round cuvettes was used in this portion of the procedure.

Pregnanediol is present in the urine as a water-soluble glucuronide. It is therefore necessary to hydrolyze it in 10 per cent by volume of hydrochloric acid, and to extract it with a suitable organic solvent. Other procedures have employed benzene, toluene, ethyl acetate, and chlorinated solvents. However, a study of a number of solvents has indicated the desirability of an aliphatic solvent such as iso-octane (2,2,4-trimethylpen-

TABLE 3. RECOVERY OF SODIUM PREGNANEDIOL GLUCURONIDATE AS PREGNANEDIOL FROM URINE

(Recovery factor of 0.420 used in calculations)

Pooled male urine sample (50 ml.)		Sodium pregnanediol glucuronidate added (mg.)	Actual recovery as pregnanediol (total recovery minus urine blank) (mg.)	Theoretical recovery as pregnanediol (mg.)	Per cent recovery as pregnanediol
	Urine blank as pregnanediol (mg.)				
1	0.017	0.25	0.080	0.105	76.2
2	0.029	0.50	0.187	0.210	89.5
3	0.128	1.00	0.382	0.420	91.0
4	0.029	1.50	0.603	0.630	95.7
					Av. recovery 88.1

tane). It has the advantage of dissolving only slightly the many colored materials which are present in hydrolyzed urine. Moreover, it does not form troublesome emulsions such as are often formed by the other solvents. Actually, in the cold, it is a poor solvent for pregnanediol. At room temperature, only about 3 micrograms will dissolve slowly in 1 milliliter of dry iso-octane. At 65°C., in water-saturated iso-octane, the solubility is about 75 micrograms per milliliter, or about 5.5 mg. in the 75 ml. used in the extraction. As not more than about 0.75 mg. should be extracted, the solubility is sufficient if warm purification reagents are employed and the separatory funnel is kept warm by shining an infra-red light upon it. Using the present extraction method, pregnanediol in the range of 0.05 to 0.75 mg. in 50 ml. of urine is separated quantitatively.

As pregnanediol is a neutral compound, it is necessary to purify it by removing practically everything else from the iso-octane and leaving it in solution. Most of the other methods of analysis remove the acidic substances by washing first with dilute NaOH and then boiling down with methanolic NaOH. This last step has been found often to lead to low re-

sults, unless great care is taken in the makeup and storage of the methanolic NaOH. This is probably due to adsorption of pregnanediol on precipitated carbonate, if present in the methanolic NaOH. Experimentally, low recoveries of known amounts of pregnanediol were noted when 50 per cent NaOH containing suspended carbonate was used. Parenthetically, in the chromatographic step, adsorption of pregnanediol occurs on carbonate. To eliminate losses due to adsorption, the extract is shaken first with 10 per cent NaOH, then with 50 per cent NaOH by weight. This last step has the same purifying effect as the precipitation with the methanolic NaOH. The 50 per cent NaOH is not as effective in removing impurities from toluene as it is from iso-octane.

Guterman (9), Astwood and Jones (4), and others have purified pregnanediol in the final stage by a single or multiple precipitation from an alcohol-water or acetone-water solution. Although this procedure gives a fairly pure product, it is necessary to work with quantities of pregnanediol exceeding 0.4 mg. For quantitative recoveries, slow, controlled precipitations are needed. The first precipitation should stand over night and each of the next two precipitations for two hours in an incubator with one-hour centrifugings between precipitations. This is an obvious disadvantage for clinical work where time is important.

To lower the limit of detection and to effect a more rapid purification, use is made of the chromatographic technique. Merck's U.S.P. precipitated calcium carbonate is an excellent chromatographing material. It affords good separations and is not as sensitive to moisture absorption from the air as is alumina. The addition of three parts by weight of Johns-Manville Celite 545 to one part of $CaCO_3$ gives a fast-flowing, fairly non-channeling mixture.

It has been found that androsterone and dehydroisoandrosterone, two of the neutral 17-ketosteroids of androgenic importance, as well as cholesterol and other chromogens, are removed by the toluene washing. Eleven grams of the chromatographing agent can retain about 2 mg. of pregnanediol, so that it will be eluted in the proper fraction.

It is advisable to check the chromatographing procedure with authentic pregnanediol before performing the urine analysis. The chromatographing agents sometimes vary somewhat in their retention of pregnanediol, making it necessary to change the eluting procedure slightly. More or less toluene wash may be required before eluting. A blank should be determined by running the pure solvents through the entire procedure.

The residue left after evaporation of the eluate is further purified from a small amount of gummy material by washing and decantation twice with 1 ml. of dry iso-octane at room temperature. The crystalline pregnanediol residue is not easily dislodged from the flask when the iso-octane is re-

moved carefully with a fine-tipped pipette or syringe. Before washing by decantation, the residue may contain as much as 50 per cent non-pregnanediol impurity, as determined colorimetrically, particularly where the original pregnanediol concentration is low.

The amount of pregnanediol can be estimated either by weighing, or by a color reaction. Although it is nonspecific, the yellow color formed in concentrated sulfuric acid by pregnanediol is quite sensitive and has been used by a large number of investigators. Quantities of 0.01 to 0.5 mg. in 10.0 ml. of acid are determined readily.

Estimation of pregnanediol by weighing necessitates larger quantities than in the colorimetric method, with the consequent treatment of large amounts of urine if the titer is low. It is no more specific than the colorimetric method.

The spectral curve of this yellow color has been measured by the use of a Beckman spectrophotometer at wavelengths between 330 and 460 millimicrons. The curve is apparently characteristic for pregnanediol, and so can be used as a means of identifying pregnanediol and estimating its purity. Many other compounds give a visually similar color but the curves are different.

The purity of the residue can be checked by the melting point, but in the present procedure there is usually not enough for both that and the estimation. It can be noted whether or not there are any colored materials on the white pregnanediol. The concentration of the pregnanediol is best calculated from the peak in density at 425 millimicrons.

The yellow color obtained is influenced by the temperature, time, and amount of water in the sulfuric acid. About the same intensity of color is obtained in five minutes at 100°C. as in an hour at 25°C. A temperature of 25°C. is preferred, because it has been found that some of the other materials which are sometimes present increase their color formation at a greater rate at the higher temperature than does pregnanediol. The color increases with time, rapidly at first, but very slowly after about twenty-five minutes at 25°C. Best reproducibility is obtained at thirty minutes. Results are erratic at one hour, probably because of side reactions. The presence of water lessens the formation of color, but with up to 5 per cent of water added to concentrated sulfuric acid, the effect is slight. The error can be eliminated by using the same batch of acid for standardization as for the actual determination.

From the preceding data, we feel that this method compares favorably in its accuracy with any of those previously proposed for the recovery of free pregnanediol from the urine. The simplicity of the procedure itself and the short time required for its completion appear to be distinct advantages. The use of aliquots in the range from 25 ml. to 100 ml. reduces prolonged extractions with bulky quantities. This feature is also of impor-

tance to subjects being studied, who are able to send 4-oz. aliquots of 24-hour urine samples to the laboratory rather than transport total 24-hour volumes. Other recent investigators (5, 8, 9, 10, 11, 12, 13), working with modifications of both basic procedures, recovered from 64 to 88 per cent of pregnanediol when pregnanediol was added in amounts of 0.2 to 0.5 mg.; and recovered from 0 to 83 per cent as pregnanediol when sodium pregnanediol glucuronidate was added in amounts of 0.4 to 1.0 mg.

SUMMARY

A new and simplified technique for the determination of free pregnanediol has been presented. This method possesses the advantages of an improved extraction agent, simplicity of technique, and the short time required for its completion. Recovery data are felt to be comparable in every way to those obtained by other methods.

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STUDIES ON THE MECHANISM OF THE SPERMATIC RELEASE OF THE MALE NORTH AMERICAN FROG (*RANA PIPIENS*)

II. THE ROLE OF THE PITUITARY, ADRENALS AND TESTES*

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THE mechanism of the gametokinetic response of the male *Rana pipiens*, although under active investigation in many laboratories, is still not clear. Li and Chang have shown that acetylcholine given in fairly large dosage will block the reactivity of male *Rana nigromaculata* and *Bufo bufo asiaticus* to both gonadotropins and adrenalin (1, 2). Following the dosage schedule of acetylcholine suggested by these authors and using the male *Rana pipiens*, profound depression of the activity of the frog was encountered, to a point approaching a shock-like state. The question then arose as to whether the nonreactivity of the animal was due to the overall depression of bodily function or to a specific inhibitory effect. Clark, Greenblatt and West have likewise demonstrated an inconstant blocking effect of a variety of adrenolytic drugs on the activity of gonadotropins and adrenalin in male *Rana pipiens* (3). Their results have been confirmed at this laboratory. Noteworthy is the fact that no one agent would invariably inhibit gametokinesis. These inconstant patterns of reaction, together with the known variation in the sensitivity of individual frogs to specific drugs, make the interpretation of these results difficult. That hypophysectomy fails to block the activity of adrenalin or gonadotropins is by now well established (1, 4, 5). This observation rules out the possibility that the adrenalin reaction is due to the effect of this drug on the pituitary with the resultant release of gonadotropins.

Sulman postulated a reverse chain reaction suggesting that gonadotropin may provoke the release of epinephrine. He showed, however, that adrenalectomy failed to inhibit gonadotropic activity (6). Moreover, it is well known that gonadotropic activity in the male *Rana pipiens* is constant

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throughout the year, whereas adrenalin reactivity passes through a spring and summer period of areactivity. These observations would clearly indicate that gonadotropic activity is not dependent upon epinephrine reactivity.

In addition to epinephrine and the gonadotropins, it has been shown that the male *Rana pipiens* will occasionally react to adrenal cortical extract, such as Upjohn's Lipo-Adrenal Extract, as well as to the specific steroid dehydroisoandrosterone (5).

These results to date, although adding data of value, still leave unexplained the basic physiology of the ejaculatory reaction in male amphibia.

It is the purpose of this communication to present further observations on the pathways and mechanisms by which the various endocrine substances evoke their reactions.

MATERIALS

All of the *Rana pipiens* used were sexually mature adult males weighing 25 to 30 grams. They were obtained from commercial sources, used only once soon after receipt, and then discarded. All experimental observations were recorded on large numbers of animals in parallel, to avoid individual variation in sensitivity. Details of the technique have been reported previously (4).

PATHWAYS OF THE RELEASE OF THE SPERM

The anatomy of the genito-urinary system of the *Rana pipiens* has been well described by Rugh (7). The testes are lateral to and attached to the kidneys by their membranous mesenteries, in which run the efferent tubules for the sperm.

In the male *Rana pipiens* the spermatic excretory tubules enter the kidney and drain into the renal glomeruli, from which point the urinary and spermatic pathways are one and the same. The ureters in the frog drain directly from the kidneys into the cloaca. The urinary bladder functions as a reservoir in which fluids are stored by regurgitational flow.

Rugh, in his monograph, describes a terminal expansion of the ureters just prior to their entrance into the cloaca, which he calls seminal vesicles and attributes to them a storage function.

Although sperm may undoubtedly be demonstrated within these slightly expanded saccular bodies, in our opinion they do not serve as a storage reservoir in the same sense as do the analogous structures in man, for reasons to be presented. After the administration of a reactive dose of hormone, seminal fluid bearing sperm can be seen to stream across the vasa efferentia and drain into the kidney. They can (within fifteen to twenty minutes) be demonstrated in the renal glomerular spaces and tubules. Pas-

sage through the ureters into the cloaca and reflux into the bladder occurs almost at once.

When the ureters are ligated just below the kidneys gametokinesis is totally blocked, precluding, it would appear, the possibility of any storage of sperm below the level of ligation. On the possibility that the storage of sperm occurs in these structures for only a limited period of time, positive reactions were induced. At the height of the reaction the ureters were ligated just below the kidneys and the frogs followed until negative cloacal smears were obtained. Reactive doses administered at this time failed to evoke the release of sperm, whereas controls showed strongly positive responses.

These results are seemingly incompatible with the existence of storage reservoirs of sperm below the level of the kidneys.

PATTERNS OF REACTION

The gametokinetic response in these amphibia follows a fairly fixed pattern, irrespective of the stimulatory agent used. When an adequate reactive dose of epinephrine or gonadotropin is administered subcutaneously to a frog, approximately fifteen to twenty minutes later a few spermatozoa can be identified in the cloacal fluid. Once the reaction starts it becomes maximal within one to two minutes, with large numbers of single and clumped sperm readily identifiable within the cloacal fluid. The reaction is sustained at this high level for two to four hours and then gradually becomes negative over the next one to two hours.

The inference is apparent that the onset of spermatogenesis resulting from an adequate stimulus consists of an ejaculatory response not unlike that found in mammalia. The protracted course of the positive reaction results from the fact that these amphibia have a common urinary and seminal excretory tract beginning in the renal glomeruli and tubules and then passing caudally through ureters to drain through the cloaca. Both urine and sperm regurgitate back into the urinary bladder in the process of gametokinesis. Hence, under ordinary conditions the persistence of the positive reaction is due to the storage of large amounts of sperm in the bladder urine. The reaction only gradually becomes negative as this reservoir is slowly depleted of its seminal content. Corroboration of this "trigger mechanism" behavior of the ejaculatory response was obtained by castration of the animal or ligation of the ureters after the onset of a positive reaction. No alteration in the strength, pattern or duration of the response occurred, indicating that the spermatic release from the testes occurred only once soon after the administration of the stimulatory agent and that sperm within the cloacal fluid persisted until the bladder content was exhausted.

The above description of a spermatic response obtains only when a stimulus adequate to produce a 4-plus reaction is given. If progressively smaller amounts of hormone are administered, a level is reached at which some animals fail to respond, while others develop a weak reaction. Such reactions, although following the pattern already described, are not only weaker but do not last as long.

SITE AND MODE OF ACTION OF HORMONES

It is, of course, obvious that any reactive substance, to produce a positive reaction, must ultimately exert its effect on the testes. In the light of the speed with which a positive reaction develops, namely, as little as fifteen minutes, a nervous pathway for the mechanism appears as an attrac-

TABLE 1

Expt. No.	Substance injected	Site of administration and degree of reaction†					
		Intra-testis		Intra-adrenal		Subcutaneous (Controls)	
		Frog #1	*Frog #2*	*Frog #1*	*Frog #2*	*Frog #1*	*Frog #2*
I	Gonadophysin 0.5 u*	++++	0	++++	++++	0	0
	Gonadophysin 0.25 u	0	0	0	0	0	0
II	Gonadophysin 1.0 u	—	—	++++	++++	0	0
	Gonadophysin 0.5 u	—	—	++++	0	0	0
III	Gonadophysin 0.5 u			++++	0	0	0
IV	Gonadophysin 2.0 u	++++	++++	++++	++++	++++	++++
	Gonadophysin 1.0 u	++++	0	++++	0	0	0

* All Gonadophysin dosage refers to manufacturer's units, Searle & Co.
† 0 = No reaction.
++++ = Production of gametokinesis.

tive possibility. However, Li and Chang have shown that pithing of the frogs, destruction of the central nervous system and transection of the cord all failed to effect the responsiveness of their amphibia (1). These results have been confirmed at this laboratory for the male *Rana pipiens*.

A humoral or endocrine pathway would be the alternative. The pituitary gland, which offered itself as a possible important link in the pathway, proved to be of no importance, hypophysectomy failing to effect the reactivity of either epinephrine or the gonadotropins. Since storage reservoirs of sperm do not exist in the *Rana pipiens*, castration inevitably blocks all reactions. For similar reasons nephrectomy, involving total adrenalectomy, blocks all reactions since it interrupts the pathways for the release of sperm.

To localize the site of action of absorbed hormones, direct injections of these reactive substances were made into various sites. The results are indicated in Table 1.

These results indicate that doses insufficient to evoke a reaction when given subcutaneously evoke gametokinesis when administered directly into the testis or adrenal, strongly suggesting these sites as the target organs of the subcutaneously administered substances.

In a previous communication it was further shown that one of the mammalian adrenal steroids, dehydroisoandrosterone, was capable of producing reactions (4). These responses were, on the whole, inconstant as well as weak. However, it furthered the suggestion that the site of action of epinephrine might be on the adrenal, evoking some steroidal secretion. Adrenalectomy then should block the adrenalin response. Unfortunately, in the frog, as has been shown, no discrete adrenal gland is found (7). Cells that are present are found distributed subserosally along the midline of the ventral surface of the kidney as well as in prolongations down into the renal substance. Destruction of these cells by some mechanical means without interference with the vasa efferentia and renal tubules was obviously a problem of some delicacy. Angerer and Angerer (8), and Sulman (6) report the successful extirpation of these cells by use of electrocautery, and Li and Chang by mechanical excision (1). We have used a variety of technics including coagulation by electrocautery, surgical excision, excision with electrocautery and destruction with silver-nitrate cautery, and have achieved considerable if not almost total removal of the cortical cells. No loss of reactivity to adrenalin or gonadotropin was noted. Serial section studies of the kidney-adrenal preparations left no doubt that although at least 80 per cent of the cortical cells appeared destroyed, scattered nests of apparently vital cortical cells could still be found within the deep recesses of the renal parenchyma. More vigorous efforts to destroy these persistent foci led to loss of the entire reactivity of the frog, probably due to the severe injury to the renal tubular system. It is likely that such drastic measures may have mechanically interfered with the excretory pathways of the sperm. This assumption is supported by the fact that even minimal cauterization of the mesentery carrying the vasa efferentia, such as might be encountered in drastic treatment of the adrenal directly, was sufficient to block all reactivity. For these technical reasons the results of adrenalectomies cannot be considered as having critical value in any elucidation of the mechanism of gametokinesis.

However, in the course of adrenalectomizing these amphibia, it was noted that many frogs were reacting postoperatively to the operation alone. These results suggest that release of some adrenal steroid or steroids may have occurred through the mechanical stimulation produced by the surgery. Nonspecific stress, such as crushing injury to the extremity or application of an irritant caustic agent to the skin, failed to evoke a reaction.

In view of the possibility that endogenous adrenal steroids may evoke a

reaction, macerates of the kidney including the adrenal cells, or macerates of excised adrenal fragments were administered to male frogs. The results follow:

Pulp administered to each frog	Reaction	
	No. positive	No. negative
3 adrenals	8	3
12 adrenals	5	1
50 adrenals	3	1

It is apparent that some substances within the adrenals are capable of evoking a reaction. The adrenal macerates failed to evoke reactions during the spring and summer months. It is of interest that the adrenal macerates parallel the adrenalin reaction in regard to this seasonal variation (4). These observations suggest that adrenalin may indeed be evoking its response through the release of adrenal steroidal substances. Depletion of these substances during the summer may be responsible for the loss of the adrenalin reactivity.

Fractionation of this adrenal macerate into water-soluble and lipo-soluble fractions indicated that the reactivity resided in the lipid fraction. Further efforts to isolate and identify this substance have to date been unsatisfactory, largely due to the limits imposed by the minute amount of substances available for chemical processing.

Parallel experiments, using testes macerates in place of adrenal tissue, failed to evoke reactions. A word of caution is in order at this point. Many seeming reactions obtained by the administration of testes macerates were found later to be due to minute contamination of the amphibia's skin by the tissue macerate harboring innumerable sperm. Most meticulous precautions and controls revealed that these testes substances were without effect.

SUMMARY AND CONCLUSIONS

To date, the exact mechanism by which spermiation is produced in the male *Rana pipiens* has not been elucidated. All the evidence is against the intermediation of the nervous system or the pituitary gland. The present investigations strongly suggest that adrenalin reactivity in these amphibia is mediated through the adrenal gland. The present results would appear to suggest that steroidal substances found in this organ may be the ultimate substances which effect the release of sperm from the testes. It is hypothecated that adrenalin acts by its effect on adrenal cortical steroids,

and gonadotropins act by their direct effect on the testes. The published reports of adrenalectomy failing to block the effect of adrenalin, although possibly correct, must be viewed with some doubt since, in our hands, post-adrenalectomy serial section study of the kidney-adrenal preparations always disclosed deeply situated preserved nests of adrenal tissue. Destruction of these nests always seriously injured the kidneys and seminal tubules. The precise mechanisms by which substances cause the passage of sperm from the testes to the cloaca is still unknown.

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TOPICAL ESTROGENS: CLINICAL EFFECTS AND SIDE ACTIONS

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SINCE Zondek (1) first showed that estrogens could pass through the intact skin of a mouse, his work has been repeatedly confirmed. In a fat-solvent vehicle he produced effects, weight for weight, equal to those obtained by subcutaneous injection (2).

Local estrogenic therapy has been successful in senile vaginitis (3) with a minimum of systemic effects. Similar results have been found in the vulvovaginitis of children (4).

Speert (5), using daily applications of an alcoholic solution of estrogens of a potency of 0.5 mg./cc., showed a fourteen-fold increase in the size of the treated nipple as compared with the untreated contralateral nipple in young male rhesus monkeys. He concluded that estrogens can act directly on responsive tissue. Production of such unilateral effect would rule out the possibility of mediation through the pitutitary.

Bloch (6), Sulzberger (7) and others noticed the special susceptibility, familial or otherwise, of certain skin areas to an altered androgen-estrogen ratio. In patients with only this local susceptibility it is important not to disturb normal ovarian function by systemic hormonal therapy. In these cases local treatment with estrogenic substances provides high concentration with minimum systemic effects (8).

Drant (9) and Goldzieher (10) found systemic and topical estrogens of value in the control of refractory menopausal and senile dermatoses.

Thirty-one men and 52 women have been studied over the past four years, using topical estrogens exclusively. Dermatoses studied have included those in which :

a) Excess male hormone is a prime factor:
   Acne vulgaris, seborrhea oleosa and male-pattern baldness, in both men and women (11).
b) A low estrogen level is etiologic:
   Keratoderma climactericum, menopausal pruritus vulvae and ill defined scaly and erythematous dermatoses in menopausal women (9).

Since a guide to the efficient clinical management of dermatoses with

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topical estrogens in high dosage may be useful and since there is a great individual variation and tolerance to estrogens, this presentation of the dosages and the effects due to topically applied estrogens is submitted.

Some unexpected local actions as well as anticipated systemic actions have been noticed.

METHODS AND MATERIALS

The following laboratory studies were made in a majority of these patients: urinalysis, B.M.R., blood count, sedimentation rate, blood sugar, serology and cyclic vaginal smears. Only significant laboratory data will be mentioned.

Estrogens in vanishing cream and in 70 per cent ethyl alcohol were applied every eight hours for six weeks. This schedule was reduced to two applications daily for the succeeding four- to six-week, period.

Dosage was varied according to the clinical effects produced. A glass rod, applicator (cotton), or stick was used to apply the solution. Extra attention was given to stubborn areas. The average daily dose consisted of 4 cc. of alcoholic lotion or 4 grams of vanishing cream, with estrogens. This amount of material was adequate for the treatment of an area about 6 inches square.

The following materials were employed in this study:

A. 1.25 mg./Gm. of conjugated estrogens[1] (equine) in vanishing-cream type base.
   2.5 mg./Gm. of conjugated estrogens (equine) in vanishing-cream type base.
   1.0 mg./cc. of conjugated estrogens (equine) in 70 per cent ethyl alcohol.
   2.5 mg./cc. of conjugated estrogens (equine) in 70 per cent ethyl alcohol.
B. 1.25 mg./Gm. of estrone[2] in flesh tinted vanishing-cream base.
   1.0 mg./cc. of estrone in flesh tinted lotion.
C. 0.5 mg./Gm. of ethinyl estradiol[3] in vanishing-cream base.
   0.125 mg./cc. of ethinyl estradiol in 70 per cent alcohol.

Whereas in the normally menstruating woman there is a premenstrual fall in the level of circulating estrogens, the continuous application of these materials provided a uniform supply of estrogens to the treated areas.

Treatment of severe and advanced conditions was started by applying the alcoholic lotion for four to six weeks, followed by use of the cream as the clinical condition improved. As soon as clinical improvement became

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[1] Special potencies of "Premarin" Lotion and Ointment were supplied through the courtesy of Dr. J. B. Jewell, Medical Director, Ayerst, McKenna and Harrison, Ltd.

[2] Special supplies of "Sharestrin" Cream and Lotion were made available by Dr. Askue, of Sharp and Dohme, Inc.

[3] "Estinyl" Lotion and Cream were kindly supplied by Dr. George Babcock, Jr., of the Schering Corporation.

evident, as indicated by decreased oiliness in the acne, or reduced erythema in other dermatoses, the dosage was sharply reduced.

No other local or general measures were used. No diet restriction was enforced.

Controls

Acne vulgaris is notorious for spontaneous remission and exacerbation. Standard methods of treatment usually result in from 80 to 90 per cent good results.

In order to avoid the complication of spontaneous cure, cases were chosen which satisfied these criteria:

1. Disease over five years' duration.
2. Relapse or failure to improve on diets, salves and lotions, Alpine-ray and roentgen-ray therapy, vitamins and vaccines. All subjects had tried several or all of these measures.
3. Severe cystic, scarring, or keloidal types of acne, generally known to be most indolent.
4. Generally increasing severity and extent of the acne process at the time the patient was first seen.

Applications of estrogens were made to the face only, leaving the chest and back lesions, when present, untreated. In all cases in which the face improved the chest and back remained unchanged.

In alopecia prematura and scalp seborrhea, cases were chosen that failed to respond to a variety of local and general measures for a period of several years.

The same was true of the cases of keratoderma, *e.g.*, H.S. (Figs. 1 and 2), in whom a remission was induced for the first time in twenty-two years.

Early withdrawal from treatment was followed by recrudescence of the eruption within a short time. Adequate treatment, followed by a small maintenance dose, produced satisfactory prolonged remissions.

CASE REPORTS

Case 1. R. H., an 18-year-old, white single girl, had severe cystic acne vulgaris.

History: She had had a papulo-pustular and cystic acne of the cheeks, chin and forehead for five years. All standard measures of treatment, including roentgen-ray therapy, failed to improve her skin. Premenstrual exacerbation was common and severe.

Treatment: This was begun with Premarin Cream (2.5 mg./Gm.). Applications of 2 Gm. to the face three times daily were increased up to four times daily for two months, gradually tapered to a maintenance dose of once daily for two months, and then reduced to 1 Gm. every third day for four months.

Clinical course: Within twelve weeks oiliness of the skin was sharply reduced. Old lesions were healing and new ones were small, superficial, and involuted rapidly. About sixteen weeks later, remission of the acne was complete. The patient was seen at monthly intervals and ordered to apply 1 Gm. to her face every third day. Her regular menses

FIG. 1

FIG. 2

FIG. 1. *H.S.*, a 60-year-old woman, with keratoderma of both palms, of twenty-two years' duration.

FIG. 2. Same patient after four weeks of topical application of high potency estrogen cream. (Reproduced from Block, C., and Shapiro, I.: Keratoderma climactericum treatment with local application of estrogens, *Am. Pract. & Digest Treat.* **1**: 1076 (Oct.) 1950. Courtesy of J. B. Lippincott Co., Philadelphia.)

failed to appear six months after beginning treatment. She then stopped ointment for one month. She resumed maintenance dosage again and when advised to cease all medication six weeks later, she noted intermenstrual "spotting" for a few days. Her acne has remained in remission for twelve months. Her menses are regular. Severe premenstrual tension and pain, which formerly gave her great distress, has vanished.

Case 2. J. M., (Fig. 3) a white single girl, had cystic keloidal acne vulgaris.

FIG. 3. *J.M.*, an 18-year-old girl with disfiguring cystic and keloidal acne of the face and back, of five years' duration, unaffected by diets, vaccines, Alpine or roentgen rays.

History: She had had a keloidal and cystic acne of the face, chest and back for five years. Previous treatment, using roentgen rays, lotions and salves, gave no relief.

Physical examination. She was a tall, flat-chested, narrow-hipped, hyperactive girl with marked papules, pustules and redness about the face, chest and back. The scalp margins showed large red cysts, oiliness, and pitted and keloidal scars. The face was most severely involved.

Treatment: This was begun with Premarin Lotion, 1 mg./cc. She applied 2 cc. to her face daily and increased this dose to 4 cc.

Clinical course: Her eruption subsided markedly in six weeks. She then used 2 cc. daily for one month. The severe grade of oiliness now disappeared and she began to note itching about the cheeks. Sharestrin Cream was begun four times a day. This gave rise to itching, so the dose was reduced to once daily (total, 2 Gm.). Itching subsided. Patch test reactions to both Premarin Lotion and Sharestrin Cream were negative. All large pustules and cysts disappeared (Fig. 4). Small premenstrual flareups were still noted.

The erythematous scaling patches in her scalp margins appeared unchanged. Her menses were regular and unchanged since starting therapy. No other measures were used. She worked regularly and maintained her usual activities and interests. The less severely involved chest and back were untreated and showed no change.

FIG. 4. *J.M.* after ten weeks of topical therapy with estrogen lotion. Remission was induced on the face but the back was unaffected. Oiliness decreased and the skin became paler, softer, and of more feminine texture.

Case 3. M. A., a 17-year old boy with scarring and pustular cystic acne, is listed here because Figures 5 and 6 show the results obtained with estrogen lotion.

DISCUSSION

Certain dermatoses appear favorably influenced by local estrogen application of high concentration. Insufficient comparative experience with the different estrogenic preparations used does not allow conclusions to be drawn as to their relative value.

More reactions[4] are reported with Premarin because more patients were treated with it.

An estrogenic alcoholic lotion appeared to be about four times as effective clinically as a comparable preparation in a cold-cream vehicle.

[4] Due to the frequency of systemic side reactions from the use of ethinyl estradiol lotion, 0.125 mg./cc., we have reduced its strength to 0.05 mg./cc., with fewer absorption phenomena.

➳

FIG. 5. *M.A.*, a 17-year-old boy with a scarring and pustular cystic acne of five years' duration. Vaccines, diets, Alpine and roentgen-ray therapy had been tried previously.

FIG. 6. *M.A.*, six weeks later, after using estrogen lotion. All lesions were involuting, no new ones had appeared, and the skin was drier.

FIG. 5

FIG. 6

TABLE 1. ACNE VULGARIS. TOPICAL TREATMENT WITH ESTROGEN CREAM

	Female	Male
Number of patients	16	10
Age	17– 70	18– 30
Duration of acne (years)	4– 22	3– 15
Total dosage (mg.) of estrogen, 2.5 mg./Gm.	105–700	150–650
Duration of treatment (months)	1– 7	1– 6
Results. Excellent	10	6
Good	2	2
Fair	2	1
Therapy discontinued	1	1
Results unusable	1	

TABLE 2. CHRONIC ACNE VULGARIS IN FEMALES: TOPICAL TREATMENT WITH ESTROGEN LOTION

Number of females	26
Age	17– 40
Duration of acne (years)	5– 18
Total dosage (mg.) of estrogen	60–1,200
Duration of treatment (weeks)	6– 16
Results: Good	15
Fair	2
Retreated (2 fair) (2 good)	4
Side actions:	
Local pruritus	4
Withdrawal bleeding	1

TABLE 3. CHRONIC ACNE VULGARIS IN MALES: TOPICAL TREATMENT WITH ESTROGEN LOTION

Number of males	18
Age	17– 45
Duration of acne (years)	5– 30
Total dosage (mg.) of estrogen	240–1,400
Duration of treatment (weeks)	6– 20
Results: Good	10
Fair	3
Retreated (good)	2
Side actions	
Pruritus	1
Gynecomastia	2

TABLE 4. PREMATURE BALDNESS AND SEBORRHEA: TREATMENT WITH TOPICALLY APPLIED ESTROGEN LOTION

	Female	Male
Number of patients	4	3
Age	25– 40	24– 29
Duration of symptoms	3 mo.–4 yrs.	1– 8 yrs.
Total dosage (mg.) of estrogen	120–240	690–1,200
Duration of treatment (months)	1– 3	6– 12
Results: Cessation of hair loss	4	2
Frequent relapses		1

TABLE 5. KERATODERMA CLIMACTERICUM AND PRURITUS VULVAE ET ANI: TOPICAL TREATMENT WITH ESTROGEN CREAM

	Keratoderma	Pruritus vulvae
Number of patients	3	3
Age	42– 60	36– 54
Duration of symptoms	2– 22 yrs.	9 mos.–2 yrs.
Total dosage (mg.) of estrogen cream	105–120	150–225
Duration of treatment (months)	1– 2	1– 2
Results:		
Good	2	3
Side actions:		
Dermatitis	1	0

In 10 per cent of cases clinical improvement appeared to coincide with local pruritus. Further study to explain this is in progress.

Eczematous skin is much more prone to sensitization. Topical estrogens would seem of limited value in these cases.

The gynecomastia which was produced was harmless, short-lived and reversible by withholding estrogens and/or giving testosterone. There were no sequelae.

Withdrawal bleeding, "spotting," and delayed menses disappeared when treatment was stopped.

No change was noted in libido, pigmentation, hirsuties, size and frequency of erections, or coitus.

Vaginal cornification studies are in progress to explore these relationships.

SUMMARY

1. Eighty-three patients (31 men and 52 women) have been treated with topical estrogens over a period of four years.

2. Clinical conditions chosen included acne vulgaris, seborrhea oleosa and male-pattern baldness in men and women, keratoderma climactericum, and menopausal vaginal pruritus.

3. Results of treatment and dosages used are described as guides in management.

4. In 10 per cent of cases local pruritus was associated with clinical improvement.

5. In appropriate clinical conditions topical estrogens produce desirable local effects before systemic changes appear.

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Letter to the Editor

USE OF TAPAZOLE TO INCREASE I^{131} CONCENTRATION IN EUTHYROID PATIENTS

To the Editor:

The antithyroid drug, 1-methyl-2-mercaptoimidazole (Tapazole, Lilly) was administered to 35 patients with normal thyroid function. Tracer doses of I^{131} were given both before Tapazole and forty-eight hours after discontinuing the drug. Thyroidal concentration of I^{131} was increased an average of 28 per cent in 27 of the 35 patients after one month's administration of Tapazole in a dose of 45–60 mg. daily. There was no palpable increase in the size of the goiters and no alteration of clinical thyroid activity. PBI determinations in 5 patients were unchanged. The biologic half-life of the I^{131} in the Tapazole-treated thyroids was not significantly shortened in 4 cases so studied.

Astwood (1) has previously noted the profound block that 1-methyl-2-mercaptoimidazole exerts on thyroid concentration of I^{131}. The "rebound phenomenon" after the drug is stopped, might be expected to be more marked than that noted following propylthiouracil. This might account for the relative inability of propylthiouracil to induce increased uptake of I^{131} in euthyroid patients (2).

Although there was no gross evidence of increase in the size of the goiters, we believe that the functional "iodide space" was enhanced in these cases. This is somewhat substantiated by the rapid discharge of I^{131} following thiocyanate administration (3).

We have used this procedure in the preparation of patients for treatment with I^{131}. It is of particular value in those patients who have severe cardiac disease and who are to have a medical thyroidectomy (4).

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The Endocrine Society

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